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# Major Histocompatibility Complex Control of T Cell Recognition

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Our understanding of the way in which the class I and class II cell surface glycoproteins encoded by the major histocompatibility complex (MHC) control T cell function has changed dramatically in the last five years. The phenomenon of MHC restriction—that T cells recognize foreign antigens only in the context of MHC molecules—has been known since the early 1970s. This breakthrough series of discoveries was followed by 10 years of insightful, exciting, and sometimes very complex experiments. These experiments advanced our understanding of the interplay between T cell recognition and MHC products, but the complexity of the experimental systems frequently meant that the interpretations were controversial. A number of technical innovations improved the situation during the 1970s. These innovations include the development of T cell cloning procedures and the more widespread use of monoclonal antibodies rather than conventional anti-

sera. Also, we now know what the T cell receptor is actually composed of in terms of its component polypeptide chains, and we have a great deal of information about the T cell receptor variable α and β chains from DNA sequencing. Of greater impact than these innovations has been the conversion of a number of other lines of work in the past five years which has convinced most researchers in the field that degraded, peptide forms of foreign protein antigens are recognized in association with the MHC products by both class I and class II restricted T cells. Progress has been so rapid that today there are many examples of peptides 8 to 20 amino acids in length which provide the foreign antigen epitope for T cell clones immunized against native protein antigens. In most instances only the peptide fragment and not the native or intact protein antigen can be recognized in association with the MHC. Coupled with this recent information is

the demonstration that purified class II molecules in detergent solutions can bind their corresponding antigenic peptides. Most recently has come the structural analysis of crystals of a human class I glycoprotein. This work yielded a picture of the MHC class I molecule and immediately provided not only sites on which to hang MHC polymorphisms but, in addition, an obvious peptide binding site.

Today most immunologists and cell biologists think of the MHC class I and class II glycoproteins as peptide binding and transport systems designed to bring fragments of foreign antigens to cell surfaces for surveillance by the T cell repertoire. In the case of a healthy individual, T helper cells are scanning class II bearing cells for novel peptide–class II complexes while cytotoxic T lymphocytes (CTLs) are scouting for novel peptide–class I complexes. In order to keep the helper and cytotoxic arms of immunity separate, one interesting suggestion is that the intracellular sorting of class II molecules in B cells and macrophages allows them to interact specifically with the peptides derived from exogenous proteins that have entered the cell by endocytosis. According to this view, class I molecules follow different intracellular routes from class II molecules and pick up the degradation products of endogenously synthesized proteins.

MHC molecules are also critically involved in shaping the T cell receptor repertoire. During T cell ontogeny in the thymus, the MHC molecules of the thymic epithelial cells are involved in selecting which T cell receptors are exported to the periphery: epithelial class II glycoproteins select the class II restricted repertoire and epithelial class I antigens select the cytotoxic T cell repertoire. The evidence for this view of T cell–MHC interactions is summarized below and some of the implications for T cell reactivity are discussed.

## DISCOVERY OF MHC RESTRICTION

### T Cell–B Cell Interaction

In the late 1960s a number of research groups reported that there was a requirement for more than antigen and B cells to induce a good antibody response. It became apparent that lymphocytes from the thymus—T lymphocytes, which were not themselves precursors of antibody-forming cells—could synergize with B cells to "help" them produce large amounts of high-affinity antibody. The classic "hapten–carrier" experiments of Mitchison and colleagues showed that the T helper cell could recognize a determinant on a carrier, such as bovine gamma globulin (BGG), and deliver help to a B cell specific for a hapten, such as dinitrophenyl (DNP), as long as the hapten and carrier were physically linked as a DNP-BGG conjugate. These experiments naturally led to the notion of an antigen bridge between the interacting T helper cell and B cell, an idea that we now know is wrong.

The hapten–carrier system was used by Katz and Benacerraf and their colleagues to provide the first evidence that the positive interaction between T helper cell and B

**TABLE 1.** *Failure of histoincompatible T and B lymphocytes to collaborate in vivo*

|  | BGG-primed helper cells | Anti-Thy 1 treated DNP-primed B cells | Anti-DNP antibody ($\mu$g/ml) at 7 days |
|---|---|---|---|
| Experiment 1 | A/J | A/J | 220 |
|  | A/J | BALB/c | 5 |
|  | BALB/c | A/J | 15 |
|  | BALB/c | BALB/c | 120 |
| Experiment 2 | A/J(n)[a] | $F_1$ | 100 |
|  | A/J | $F_1$ | 1,750 |
|  | $F_1$(n)[a] | $F_1$ | 200 |
|  | $F_1$ | $F_1$ | 1,750 |

[a] The (n) refers to unprimed spleen cells.

*Note:* Spleen cells from unprimed or BGG-primed animals were transferred into $F_1$ hosts, which were irradiated with 600 R 24 hr later. Spleen cells from DNP-BSA-primed mice were depleted of T cells by antibody and complement lysis and transferred into the same hosts along with the antigen DNP-BGG. Mice were bled 7 days later and the serum assayed for anti-DNP antibody.

Adapted from Katz et al. (1).

cell was MHC restricted; that is, carrier-primed T cells from one strain of mouse could help syngeneic but not allogeneic B cells (1). An example of some of their results is shown in Table 1. BGG (carrier)-primed spleen cells from a strain A/J(H-2$^a$) mouse were injected into an adoptive host $F_1$(BALB/c × A/J)(H-2$^d$ × H-2$^a$) mouse and 24 hr later the animals were irradiated and injected with a source of DNP-BSA-primed spleen cells, which had been T cell depleted, and the test antigen DNP-BGG. Seven days later the mice were bled and the serum assayed for anti-DNP antibody. The results clearly showed that H-2$^a$ T cells could provide antigen specific help to syngeneic B cells but not to allogeneic, H-2$^d$ B cells. Importantly, H-2$^a$ T cells and H-2$^d$ T cells could provide BGG specific help to $F_1$ B cells. These and similar experiments demonstrated that H-2 homozygous T helper cells could interact in a carrier specific fashion with syngeneic and heterozygous B cells, and that heterozygous T cells could help B cells from either parent.

Sprent provided a very clear example of the latter result, which also showed that T helper cells in a heterozygous (H-2$^k$ × H-2$^b$)$F_1$ mouse consist of two separate subpopulations, one H-2$^k$ restricted and the other H-2$^b$ restricted (2). T cells from unprimed $F_1$ mice were depleted of B cells and macrophages and transferred with the antigen sheep red blood cells (SRBCs) into heavily irradiated $F_1$ or parental strain mice. T helper cells in the inoculum became primed by reacting with the antigen in the spleen of the recipient. These primed cells appear in large numbers as activated T cells in the thoracic duct lymph 5 days later. The ability of these activated $F_1$ T cells to provide help to $F_1$, H-2$^k$ and H-2$^b$ B cells in the production of anti-SRBC antibody was assayed by coinjection into secondary irradiated $F_1$ hosts. IgM and IgG antibody-forming cells specific for SRBCs were enu-

**TABLE 2.** *Restricted helper function of T cells from $F_1$ mice following priming in different environments*

| Irradiated hosts used for priming $F_1$ T cells | Source of B cells tested for cooperation | Anti-SRBC PFC/spleen ($\times 10^{-3}$) at 7 days | |
|---|---|---|---|
| | | IgM | IgG |
| (B6 × CBA)$F_1$ | CBA | 21.2 | 125.7 |
| (B6 × CBA)$F_1$ | B6 | 13.9 | 56.2 |
| (B6 × CBA)$F_1$ | $F_1$ | 14.7 | 89.4 |
| CBA | CBA | 30.5 | 275.4 |
| CBA | B6 | 3.9 | 9.4 |
| CBA | $F_1$ | 13.1 | 74.5 |
| B6 | CBA | 1.2 | 8.6 |
| B6 | B6 | 40.6 | 118.9 |
| B6 | $F_1$ | 7.4 | 84.7 |

*Note:* Nylon-wool-purified T cells from normal (B6 × CBA)$F_1$ ($H\text{-}2^b \times H\text{-}2^k$) mice were injected into irradiated host mice with SRBCs. Five days later activated T cells were collected from the lymph, shown to be of $F_1$ origin, and assayed for their ability to provide help to SRBC B cells by coinjection into irradiated $F_1$ hosts along with antigen. PFC assays were performed 7 days later.
Adapted from Sprent (2).

**TABLE 3.** *Positive selection of $F_1$ T lymphocytes by antigen-pulsed parental macrophages*

| $F_1$ T cells selected on | Mφ used for restimulation | Proliferation (cpm × $10^{-3}$) | |
|---|---|---|---|
| | | Unpulsed | OVA pulsed |
| Unselected | 2 | 2.5 | 44.5 |
| | 13 | 3.9 | 52.3 |
| OVA pulsed Strain 2 Mφ | 2 | 0.8 | 85.3 |
| | 13 | 1.0 | 1.8 |
| OVA pulsed Strain 13 Mφ | 2 | 0.9 | 23.4 |
| | 13 | 2.7 | 83.3 |

*Note:* T lymphocytes were obtained from (2 × 13)$F_1$ guinea pigs previously primed with ovalbumin (OVA) by purification of peritoneal exudate cells over rayon wool columns. Some cells were tested directly for responsiveness (unselected), whereas others were cultured with OVA-pulsed strain 2 or strain 13 macrophages (Mφ) for 1 week prior to restimulation.
Adapted from Paul et al. (4).

merated 7 days later. $F_1$ T cells activated by antigen in an $F_1$ environment collaborated with all three types of B cells (Table 2). The same pool of $F_1$ T cells activated by SRBCs in the $H\text{-}2^k$ environment were able to help $H\text{-}2^k$ and $F_1$ B cells but not $H\text{-}2^b$ cells, and vice versa for the helper cells activated in the $H\text{-}2^b$ environment.

## T Cell–Macrophage Interaction

Almost simultaneously with the initial experiments of Katz and Benacerraf suggesting a requirement for H-2 matching for effective T cell–B cell collaboration came the experiments of Rosenthal and Shevach (3) demonstrating that the proliferative response of primed T cells to antigen-pulsed macrophages also required sharing of histocompatibility molecules. These findings were originally made with two strains of guinea pig and they were rapidly extended to the mouse where the greater availability of recombinant strains allowed the mapping of the genes to the *I-A* region of the MHC for an antigen such as ovalbumin.

Paul et al. (4) used *in vitro* techniques to show that T cells from $F_1$ guinea pigs specific for ovalbumin consist of two separate subpopulations. Each subpopulation could be selectively expanded by antigen-dependent growth on antigen-pulsed macrophages of either parental MHC haplotype ("positive selection"). Alternatively, one proliferating population could selectively be knocked out by allowing the incorporation of bromodeoxyuridine (BUDR) and exposure to fluorescent light ("negative selection"). Table 3 shows an example of positive selection by growth of a (2 × 13)$F_1$ ovalbumin-primed T cell population on ovalbumin-pulsed strain 2 or strain 13 mac-

rophages. An example of strain specific negative selection is given in Table 4.

## CTL–Target Interaction

Last, but by no means least, was the demonstration that CTLs generated against virus-infected or chemically modified syngeneic cells were specific for a self class I antigen in addition to the foreign antigenic stimulus. The impor-

**TABLE 4.** *Negative selection of $F_1$ T lymphocytes by antigen-pulsed parental macrophages*

| OVA-pulsed Mφ in first culture | Antigen | Second culture proliferation (cpm × $10^{-3}$) to Mφs from | |
|---|---|---|---|
| | | Strain 2 | Strain 13 |
| Strain 2 | None | 1.0 | 4.9 |
| | OVA | 1.9 | 25.4 |
| | PPD | 86.8 | 55.9 |
| Strain 13 | None | 26.8 | 2.4 |
| | OVA | 102.7 | 4.5 |
| | PPD | 95.3 | 67.7 |

*Note:* T lymphocytes from OVA- and PPD-primed (2 × 13)$F_1$ guinea pigs were cultured with OVA-pulsed strain 2 or strain 13 macrophages (Mφ) for 2 days, at which time bromodeoxyuridine was added. After another 24 hr the cultures were exposed to light for 90 min, washed, and recultured with strain 2, 13, or $F_1$ macrophages pulsed with OVA, PPD, or nothing (second culture). Incorporation of tritiated thymidine was measured 3 days later (cpm). Underlined values indicate instances of specific negative selection.
Adapted from Paul et al. (4).

**TABLE 5.** *MHC compatibility requirement to observe virus specific CTL-mediated lysis*

| Effector cells[a] | Target source | Lysis (%) of macrophage targets[b] | |
|---|---|---|---|
| | | Infected | Uninfected |
| Balb/c, immune | Balb/c | 78 | 47 |
| Balb/c, normal | Balb/c | 50 | 44 |
| CBA, immune | Balb/c | 33 | 49 |
| CBA, normal | Balb/c | 47 | 40 |
| Balb/c, immune | CBA | 44 | 41 |
| Balb/c, normal | CBA | 41 | 41 |
| CBA, immune | CBA | 73 | 40 |
| CBA, normal | CBA | 47 | 44 |

[a] Balb/c ($H-2^d$) or CBA ($H-2^k$) mice were infected intracerebrally with LCMV and their spleen cells used as effector CTLs 7 days later.

[b] Macrophage target cells were peritoneal cells from normal mice infected with LCMV or uninfected, and labeled with $^{51}Cr$.

Adapted from Zinkernagel and Doherty (5).

tance of this work was that it extended the newly emerging MHC restriction phenomenology to a system that involved a very short assay system to measure the intercellular interactions and it provided a system in which one of the partners, the target cell, could be a cloned fibroblast line or any class I expressing cell line. Of equal importance to this practical difference was the novel explanation of the histocompatibility requirement that was offered by Zinkernagel and Doherty from their work on the CTL response to lymphocytic choriomeningitis virus (LCMV)-infected cells (5). Their suggestion, dubbed the "altered-self" hypothesis, was that the virus infection modified the class I antigens of the infected cell such that the CTL response was mounted to this new antigenic determinant or neoantigen on the self-encoded class I glycoprotein.

In the LCMV system, the restriction phenomenon was discovered by chance. The researchers infected a variety of mouse strains with LCMV and tested the spleen cells

of the mice 7 days later for their ability to lyse LCMV-infected L cell fibroblasts. The fibroblast line was chosen as the target for convenience. What the results showed was that only the $H-2^k$ mouse strains showed virus specific lysis of the L cell target—which is derived from an $H-2^k$ strain, C3H. Spleen cells from infected $H-2^b$ or $H-2^d$ mice were inactive in the cytotoxic assay. Aware of the histocompatibility requirements in T helper systems, Zinkernagel and Doherty then performed the key experiment summarized in Table 5. In this designed test of restriction they infected $H-2^k$ and $H-2^d$ mice with LCMV to produce CTLs and tested them on both $H-2^k$ and $H-2^d$ target cells. The results were clear-cut. Effector CTLs from infected mice were able to show virus specific killing only of the H-2 matched target cells (5).

Independently of this work on virus specific responses, Shearer and colleagues had generated hapten specific CTLs by coculturing normal spleen T cells together with trinitrophenyl (TNP)-modified syngeneic cells for 5 days. Effector cells lysed TNP-modified syngeneic targets and not unmodified targets or TNP-modified H-2 unmatched target cells (6). An example of this work is shown in Table 6, which also illustrates that the compatibility requirement maps to a class I locus. In the example shown, primed B10.D2 CTLs could lyse TNP-A.TL target cells which had a $D^d$ allele in common with B10.D2 but failed to lyse TNP-B10.BR cells which shared no alleles. This work was followed by many examples of class I-restricted CTL-mediated cytotoxicity, including the demonstration that "normal" cellular components such as the male specific antigen H-Y or any minor histocompatibility antigen could serve to generate class I-restricted CTLs in a genetically different host. Thus it was not necessary to introduce foreign components such as viruses to "alter" the MHC-encoded antigens.

## MHC-LINKED IMMUNE RESPONSE GENES

The genetic control of the immune response was first demonstrated at the level of the immunogenicity of simple amino acid polymers by Benacerraf and his colleagues (7).

**TABLE 6.** *Hapten specific CTLs are restricted by class I molecules of the MHC*

| Responder[a] T cells | Target cells | MHC alleles[b] | | | | | Lysis |
|---|---|---|---|---|---|---|---|
| | | K | I-A | I-E | S | D | |
| B10.D2 | B10.D2 | *d* | *d* | *d* | *d* | *d* | -2.4 |
| | TNP-B10.D2 | *d* | *d* | *d* | *d* | *d* | 19.2 |
| | TNP-B10.BR | k | k | k | k | k | 2.4 |
| | TNP-A.TL | s | k | k | k | *d* | 15.6 |

[a] Spleen cells from normal B10.D2 mice were stimulated in culture with trinitrophenyl (TNP)-modified autologous spleen cells for 5 days and assayed for lysis on the various target cells.

[b] Letters refer to the haplotype source of origin of the alleles in each region of the MHC. Underlined letters are shared between the responder CTL and target cells.

Adapted from Shearer et al. (6).

These workers were the first to observe that certain members of an outbred guinea pig population failed to respond to hapten derivatives of poly-L-lysine. Some individual animals failed to produce antibody or delayed-type hypersensitivity reactions. By breeding studies and the use of inbred strains of guinea pigs, it was subsequently demonstrated that immune responsiveness was under genetic control by an unknown immune response (*Ir*) gene (8). The actual position of an *Ir* gene was subsequently mapped within the murine MHC to the *I* region, between the *H-2K* and *H-2D* loci, in a series of experiments using *H-2* recombinant mice by McDevitt and his collaborators (9). It is now recognized that the murine immune response or *I* region is made up of two subregions encoding the class II glycoproteins (Ia molecules) I-A and I-E of the MHC.

The discovery of *Ir* genes and their mapping to the MHC occurred prior to the discovery that all antigens were recognized in the context of the MHC-encoded molecules of the antigen-presenting cell, that is, MHC restriction. Because assays for class II MHC-restricted T cell responses (such as delayed-type hypersensitivity and antibody production) predated the advent of assays to measure class I-restricted cytotoxic responses, the first *Ir* genes to be mapped were located in the *I* region. With the availability of assays capable of measuring target cell lysis by CTLs *in vitro*, a number of cases of *Ir* gene-controlled nonresponsiveness have been mapped to the class I loci, *K, D,* and *L* in mice (Table 7).

## Early Attempts to Explain MHC Restriction and *Ir* Gene Phenomenology

The first obvious interpretation of a dominant *Ir* gene that controls the ability of T cells to respond to a specific antigen is that the *Ir* gene encodes the T cell receptor for antigen. This idea became less attractive when experiments performed after the discovery of MHC restriction placed the *Ir* gene products in the antigen-presenting cell (B, macrophage, or target cell) rather than in the T cell and showed that *Ir* genes appeared to code for MHC molecules. The alternative explanation was to view *Ir* gene control of immune responses as a special case of MHC restriction of T cell responses. In order to do this, we need to consider the mechanisms underlying MHC control of T cell recognition.

Since the discovery of MHC restriction there have been two broad classes of explanation for MHC-linked control of T cell recognition:

1. T cells recognize epitopes on the foreign antigen and on the self-MHC-encoded marker somewhat independently. According to this view, the T cell may have two receptors, one for foreign epitopes and one for self-MHC, which can be manipulated separately.
2. T cells have one receptor that can bind the complex of foreign antigen plus self-MHC. The receptor may bind only the foreign antigen, which is held or presented by the MHC molecule in an MHC-allele specific way—the determinant selection hypothesis (10). Alternatively, the receptor may bind self-MHC "modified" by the presence of the foreign antigen—the altered-self hypothesis (11).

Evidence in favor of corecognition of a complex of MHC-restricting element and antigen came from studies on T cells expressing dual specificities, for example, H-$2^1$ + X and H-$2^2$ + Y. Kappler et al. (12) derived a class II-restricted T cell hybridoma with such properties by somatic cell fusion. They began by establishing a T cell hybridoma specific for ovalbumin restricted to I-$A^k$. To this they fused a normal T cell line specific for KLH and restricted to I-$A^f$. If the two-receptor model were correct, the product of this fusion should recognize each antigen restricted to either I-$A^k$ or I-$A^f$; in other words, antigen and MHC specificities should segregate separately. This did not occur. The dual-reactive clone derived from this fusion recognized ovalbumin restricted to I-$A^k$ but not I-$A^f$ and, conversely, recognized KLH restricted to I-$A^f$ but not I-$A^k$ (Table 8). This argued against the independent recognition of class II MHC and antigen.

Hunig and Bevan (13) demonstrated a similar effect through the analysis of class I-restricted CTL clones that had two distinct antigenic specificities. They generated normal, diploid CTL clones that were specific for one minor histocompatibility antigen (X) restricted to H-$2D^d$ and a completely different minor histocompatibility antigen (Y) restricted to H-$2K^k$. The combinations of X + H-$2K^k$ or Y + H-$2D^d$ were not recognized. In this case, the two antigens interacted with different MHC products to form equivalent or cross-reactive complexes and were recognized by the same T cell receptor; the alternative combinations of nominal antigen and MHC did not form an entity the T cell could recognize. Heber-Katz and colleagues (14) demonstrated that MHC could also affect the fine specificities of given T cell clones. They demonstrated that certain cytochrome *c* specific T cell clones could recognize both pigeon and moth cytochrome *c* presented by B10.A accessory cells, but when presented by B10.A(5R) accessory cells they recognized only pigeon cytochrome *c*. Since the pigeon and moth cytochrome *c* molecules are closely related, they were also able to define the region of the antigen which determined the MHC control. The combined data of Kappler et al. (12), Hunig and Bevan (13), and Heber-Katz et al. (14) established

TABLE 7. *Murine responder and nonresponder class I and class II alleles for various antigens*

| Antigen | Nonresponder alleles | Responder alleles |
|---|---|---|
| H-Y, male specific antigen | $K^b, D^d$ | $D^b, K^k$ |
| Influenza nucleoprotein | $K^b, D^d, D^k, L^d$ | $D^b, K^d$ |
| (Phe,G)-A-L | I-$A^s$ | I-$A^b$ |
| Bovine insulin | I-$A^k$ | I-$A^b$ |
| Hen egg lysozyme | I-$A^{b,d}$ | I-$A^{k,p}$ |
| Pigeon cytochrome *c* | I-$E^d$ | I-$E^k$ |

**TABLE 8.** *Lack of independent antigen and MHC recognition in a hybrid T cell line*

| T cell line | IL-2 Production induced by | | | |
|---|---|---|---|---|
| | $I\text{-}A^k + OVA$ | $I\text{-}A^k + KLH$ | $I\text{-}A^f + OVA$ | $I\text{-}A^f + KLH$ |
| Parent 1 | + | − | − | + |
| Parent 2 | − | − | − | + |
| Fusion (1 × 2) | + | − | − | + |

*Note:* A hybridoma T cell line reactive to ovalbumin (OVA) plus I-A$^k$ was fused with normal T cells reactive with keyhole limpet hemacyanin (KLH) and I-A$^f$. Following extensive subcloning, the fused cells were tested for their ability to secrete IL-2 in response to stimulation with OVA or KLH and I-A$^k$ and I-A$^f$ antigen-presenting cells.
Adapted from Kappler et al. (12).

that T cell recognition involves a trimolecular complex of T cell receptor, antigen, and MHC and spoke against the notion that foreign antigen and the MHC-restricting element could be recognized independently.

It is within this concept of MHC restriction that investigators began to examine the role of MHC in controlling the immune response. Specifically, how do responder alleles within the MHC control the response to a given antigen? Based on either the altered-self model of Zinkernagel and Doherty (5,11) or the determinant selection model (10), nonresponsiveness can be explained as a failure to elicit T cells specific for certain combinations of MHC products (either class I or class II) and foreign antigens. For example, $I\text{-}A^d$ and $I\text{-}E^d$ are all nonresponder alleles for the protein antigen pigeon cytochrome $c$, and $K^b$, $D^d$, $L^d$, and $D^k$ are all nonresponders for the influenza virus nucleoprotein; these combinations fail to elicit a T cell response. There are two possibilities for this failure: (a) the complex forms but there are no T cells that recognize it, or (b) the complex does not form.

The latter hypothesis states that certain combinations of MHC products and antigen cannot exist; for example, the antigen pigeon cytochrome $c$ and $I\text{-}A^d$ are an incompatible combination. According to this hypothesis, failure of a given antigen to elicit a response within a nonresponder results from defective antigen presentation by the individual's MHC products. In contrast, the first hypothesis predicts that while a given combination of MHC product and antigen is permissible and does indeed form, recognition of this pair is limited by the T cell repertoire. In other words, there exist *holes* in the T cell repertoire. These holes could result if the foreign antigen–MHC complex were to mimic a self-protein–MHC complex for the purpose of T cell recognition or as a consequence of limitations within the genomic repertoire itself. We return to this discussion after we have described the recent work on the interaction between foreign antigens and MHC restriction elements.

## ANTIGEN PROCESSING

It is currently accepted that antigen that is associated with the MHC class I- and class II-restricting elements has undergone some form of denaturation or processing to generate the determinants recognized by T cells. In many cases this processing event appears to involve the proteolytic degradation of the antigen into smaller fragments, which are subsequently able to form stable complexes with the appropriate MHC molecules.

### Processing of Class II-Restricted Antigens

The realization that native antigen undergoes alterations before it can be recognized by the T cell receptor came out of studies on class II-restricted responses. Very early on it was shown that denatured protein antigen could work as well as native antigen in recalling a delayed-type hypersensitivity reaction that was originally induced with the native antigen (15). Subsequent to this surprising *in vivo* finding came the *in vitro* demonstration of basically the same phenomenon with primed T cell populations or T hybridomas. Furthermore, the *in vitro* experiments were able to demonstrate that the T cells responsive to denatured antigen plus APC were the same clones as those able to respond to native antigen plus APC. The absolute requirement for cellular processing of the T cell antigen was first demonstrated for class II-restricted antigen. This realization resulted in part from an understanding of the function of some of the cells involved in antibody production and from the ability to use antigens that were reasonably well defined.

The importance of antigen processing evolved during the early 1960s as it became apparent that macrophages played a central role in taking up and retaining antigen during antibody formation. The known ability of macrophages to degrade soluble and particulate antigens naturally led to speculation on the involvement of cellular processing in the development of an immune response. Thus the concept of "antigen processing" was introduced into immunology. These early experiments also showed that the presumably processed, macrophage-associated antigen was in fact far more efficient in eliciting an immune response than the free form of the same antigen. However, it was not until Rosenthal and Shevach (3) had demonstrated MHC-restricted interactions between T cells and macrophages that investigators began to develop a better understanding of the involvement of antigen processing in the T cell recognition of class II-restricted an-
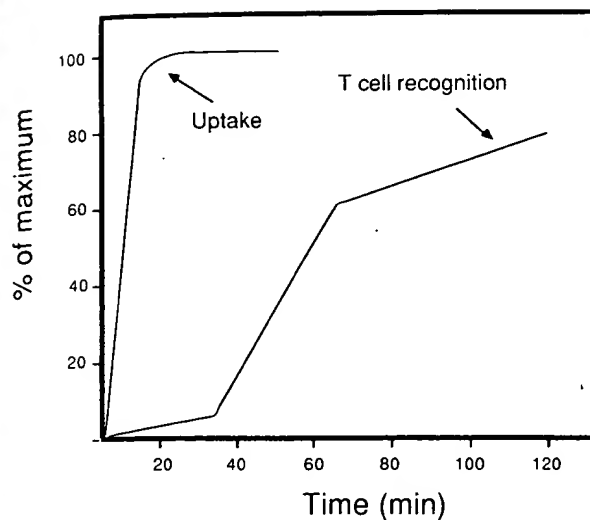
**FIG. 1.** *Listeria* uptake by macrophages and T cell recognition of *Listeria*-treated macrophages as a function of time. Adherent macrophages were exposed to heat-killed, $^{125}$I-labeled *Listeria monocytogenes* for 5 min and then unbound *Listeria* were washed away. Antigen uptake was measured as the radioactivity bound to the macrophages and T cell recognition was measured as a quantitation of T cell–macrophage binding. (Adapted from Zeigler and Unanue, ref. 16.)

tigens. It was Zeigler and Unanue (16) who clearly demonstrated that macrophages required a certain amount of time to accumulate the immunogenic form of a given antigen and that this time lag was associated with antigen processing.

The system used by Zeigler and Unanue relied on the quantitation of antigen specific binding of immune T cells to macrophages. The antigen employed was the bacterium *Listeria monocytogenes*. By radioactively labeling this antigen, the uptake of *Listeria* by macrophages and its subsequent ingestion and catabolism could be followed. They compared the uptake of *Listeria* by macrophages with the development of T cell binding activity by these cells, as shown in Fig. 1. Whereas *Listeria* binding to macrophages was maximal by 5 min, the development of T cell binding capacity required a further 30 to 60 min after the initial antigen–macrophage binding. If the mac-

rophages were rendered metabolically inactive by aldehyde treatment immediately after antigen binding, T cell binding did not develop. Fixation after the 60-min lag time had no effect on T cell association. These results were interpreted as evidence for the requirement of antigen processing in the generation of class II-restricted antigenic determinants. By subsequently using two drugs that inhibit the intracellular action of acid hydrolases, ammonium chloride and chloroquine, Zeigler and Unanue (17) provided strong evidence that antigen processing was in fact dependent on antigen degradation. Since both inhibitors interfere with lysosomal function, the experiments served to identify intracellular degradation as a key pathway of antigen handling by accessory cells.

At this stage there still remained some uncertainty as to whether processing might involve modifications to antigen in addition to the degradation events suggested by Zeigler and Unanue. These doubts were dispelled in experiments on "cell-free antigen processing" carried out by Shimonkevitz et al. (18). Their investigations made use of a combination of glutaraldehyde fixed antigen-presenting cells and a predigested form of the soluble antigen ovalbumin. They began by showing that aldehyde fixation of antigen-presenting cells that had been preincubated with native ovalbumin and allowed to process this antigen for an adequate period (more than 1 hr) did not affect their ability to stimulate a series of ovalbumin specific T cell hybridomas as measured by the release of interleukin 2. Fixation of the presenting cells prior to addition of native antigen completely abrogated T cell responses. This finding was consistent with the earlier results of Zeigler and Unanue, who demonstrated that fixation stopped antigen processing. However, as shown in Table 9, while these fixed cells failed to present native ovalbumin, they served as excellent antigen presenters for ovalbumin that had been degraded by trypsin digestion or cyanogen bromide cleavage. Live antigen-presenting cells could present degraded and whole antigen. These results strongly suggested that antigen degradation resulting in the production of peptide fragments was the necessary and sufficient step in accessory cell processing of soluble antigen.

These workers then proceeded to identify which peptides from the trypsin digest of ovalbumin were necessary for T cell recognition (19). One tryptic peptide, corresponding to ovalbumin residues 323 to 339,

**TABLE 9.** *Stimulation of ovalbumin specific T cell hybridoma by fixed versus live accessory cells*[a]

| | IL-2 release (unit/ml) in response to 1 mg/ml | | | |
|---|---|---|---|---|
| | Native ovalbumin | Denatured ovalbumin | Trypsin-digested denatured ovalbumin | CNBr-degraded denatured ovalbumin |
| Live accessory cells | 1280 | 1280 | 640 | 640 |
| Fixed accessory cells[b] | 0 | 0 | 320 | 320 |

[a] Antigen stimulation of T cell hybridomas was measured as the amount of specific IL-2 release in the presence of various forms of ovalbumin.
[b] The accessory cells were fixed by a 30-sec treatment with 0.05% of glutaraldehyde.
Adapted from Shimonkevitz et al. (18).

defined the determinant recognized by the majority of I-A$^d$-restricted, ovalbumin specific T cell hybridomas. Synthetic OVA$_{323-339}$ was equivalent to the natural fragment for T cell stimulation when presented by aldehyde-fixed or live antigen-presenting cells.

All the experimental data to this point implied that T cells recognize peptides in association with the class II MHC gene products, the Ia molecules. However, formal proof that Ia and peptide were the only elements necessary for T cell recognition came from a series of experiments by McConnell and his collaborators. Their major innovation was in the use of a synthetic lipid bilayer supported on a rigid glass surface such as a coverslip or glass bead. This bilayer, called a supported planar membrane, was developed to study the behavior of cell surface structures involved in cell–cell recognition. Purified surface glycoproteins could be incorporated within these defined model membranes. Brian and McConnell (20) began by demonstrating that class I MHC proteins in planar membranes were effectively recognized by T cells and could elicit an allospecific T cell response. Watts et al. (21) extended these findings to assess the capability of planar-membrane-bound MHC to present peptides for class II-restricted T cell activation. The choice of Ia and peptide was dictated by the earlier results of Shimonkevitz et al. (18,19). Ia-containing planar membranes were used to present trypsinized ovalbumin to one of the I-A$^d$-restricted T cell hybridomas used in the studies with the fixed accessory cells. Figure 2 shows the results of these experiments. I-A$^d$-containing planar membranes effectively presented peptide fragments to the ovalbumin specific T cells. In contrast, planar membrane alone, or planar membrane containing an inappropriate restricting element, I-A$^k$, failed to elicit any peptide specific response. Significantly, I-A$^d$-containing membranes, which are clearly unable to process antigen, failed to present the native ovalbumin, thus confirming that in this example protein degradation was an absolute requirement for T cell recognition. These results clearly demonstrated that the only features necessary for T cell recognition were the appropriate Ia molecules and peptide fragments.

## Processing of Class I-Restricted Antigens

The major breakthrough in understanding the form of the class I-associated foreign antigen resulted as a consequence of experiments designed to define the target antigens involved in virus specific cytotoxic T cell lysis. The studies by Townsend and colleagues on the CTL response to influenza virus ultimately provided conclusive evidence that the class I MHC-associated antigen was likely to exist as a fragmented form of a viral gene product.

Influenza virions consist of three polymerases as well as a nucleoprotein (NP) associated with the RNA genome. Matrix proteins underlie the host-derived lipid envelope, while two glycoproteins, hemagglutinin and neuraminidase, protrude to the outside of the virion envelope. It is the evolutionary varying surface glycoproteins that contribute to the serologically distinct grouping of influenza
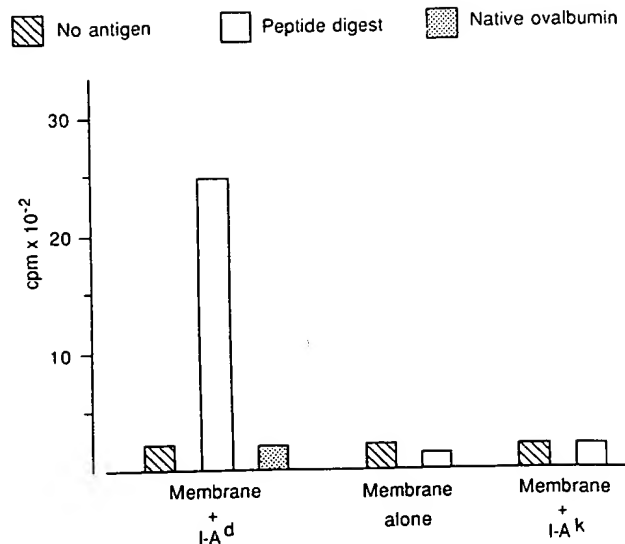


**FIG. 2.** Antigen presentation by supported planar membranes containing purified Ia proteins. Supported lipid planar membranes were made without Ia (membrane alone) and with purified I-A$^d$ or I-A$^k$. An I-A$^d$-restricted, ovalbumin specific T cell hybridoma was incubated with these membranes in the absence or presence of either native ovalbumin or ovalbumin peptide digest. The activation of the T cell was assessed by measuring the release of interleukin 2 into the culture medium. (Adapted from Watts et al. ref. 21.)

virus isolates, and it was initially assumed that these same glycoproteins would also contribute to the recognition of influenza-infected cells by CTLs. This made sense; these glycoproteins are found on the surface of infected cells naturally inserted into the plasma membrane and thus were assumed to require no special mechanism to be recognized by class I-restricted T cells. Early investigations identified two populations of influenza specific, class I-restricted cytotoxic T cells. One population was specific for certain strains of serologically related viruses (strain specific) while the second and surprisingly larger population was cross-reactive for virtually all influenza A virus isolates. At the time it was argued that these specificities resulted from recognition of variable and conserved regions on the hemagglutinin or neuraminidase glycoproteins by the respective cytotoxic T cells. However, when Townsend and Skehel (22) began to examine in detail the virus specificity of the strain specific and cross-reactive CTL clones derived as a consequence of a secondary immune response to influenza A virus in C57BL/6 mice, they found that this was not the case.

Initial experiments using a panel of influenza virus isolates from 1933 to 1947 demonstrated that subtype specific CTLs differentiate between virus strains isolated prior to 1943 and those derived after 1946. While the external glycoprotein remained closely related between the 1943 and the 1946 strains, the internal NP had undergone a significant change during that period. This suggested that the internal nucleoprotein and not the surface glycoproteins served as the CTL target antigens. To confirm this, two influenza recombinant viruses, which differed only at the
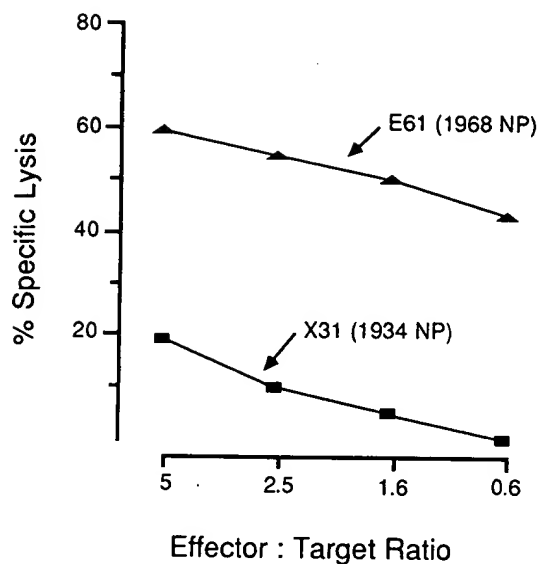
**FIG. 3.** Nucleoprotein specificity of CTL derived from influenza-virus-infected mice. A class I-restricted, influenza virus specific cytotoxic T cell line was derived from E61-infected C57BL/6 (H-2$^b$) mice and assayed for lysis of E61-infected (▲) or X31-infected (■) H-2$^b$ target cells. (Adapted from Townsend and Skehel, ref. 23.)

nucleoprotein, were used: E61, which expressed the NP from a 1968 influenza strain, and X31, which expressed the NP from a 1936 virus (23). Influenza subtype specific CTL populations derived from E61-infected mice failed to recognize the X31 recombinant (Fig. 3). These CTLs recognized the nucleoprotein gene product expressed by the E61 virus. Ultimate proof that NP represented the target antigen required for influenza strain specific CTLs came from experiments using transfected L cell fibroblasts that coexpressed the *NP* gene along with the D$^b$ restricting element necessary for effective antigen presentation.

By this stage a number of investigators had shown that other conserved internal influenza proteins, such as polymerase and matrix, were also recognized by CTLs. These results immediately raised questions regarding the

form in which viral products were recognized by the CTLs. For example, the nucleoprotein is made on free ribosomes and contains no identifiable sequence that would allow its translocation across membranes to reach the cell surface.

An early study by Gooding and O'Connell (24) had demonstrated that murine CTLs raised against SV40 virus-infected cells could recognize L cells that expressed only a part of the gene encoding the SV40 middle T antigen. Townsend and colleagues made use of this approach to define the regions on the nucleoprotein recognized by two influenza specific cross-reactive CTL populations—those from CBA mice (H-2$^k$ restricted) and those from C57BL/6 mice (H-2$^b$ restricted) (25). L cells were derived that had been transfected with truncated forms of the nucleoprotein gene. The results of CTL recognition and lysis for L cells expressing 1968 *NP* gene fragments coding for amino acids 1 to 130, and 1, 2, and 328 to 498, as well as the intact 1968 *NP*, are shown in Table 10. The cross-reactive CTLs from CBA mice recognized a determinant contained within the N terminal 130 residues of the nucleoprotein, while the CTL from C57BL/6 mice recognized a determinant found in the C terminal portion beyond residue 328. The significance of these results was the finding that the two NP fragments could reach their destination on the surface of the transfected cells and be recognized by CTLs completely independently of each other. The intact protein was clearly not required for efficient class I-restricted T cell recognition. One possible explanation for this was that the nucleoprotein was actually degraded within the cytoplasm of infected or transfected cells, producing short fragments that were the form of antigen recognized in association with the class I MHC molecules.

Townsend and colleagues tested this prediction by attempting to use synthetic peptides to directly sensitize uninfected cells for NP specific lysis (26). L cell targets were once again transfected with the gene expressing the appropriate restricting element (D$^b$). The specificity of the C57BL/6 substrain specific T cell clone used in this experiment had been previously mapped to a region that included NP residues 365 to 380. As shown in Fig. 4, a

**TABLE 10.** *Influenza nucleoprotein specificity of C57BL/6 and CBA cytotoxic T cell lines*

| NP gene segments used for transfecting L cells[a] | | CTL lines[b] | |
|---|---|---|---|
| | | C57BL/6 | CBA |
| 1968 NP$_{1-498}$ | 1————————498 | + | + |
| 1968 NP$_{1-130}$ | 1————130 | – | + |
| 1968 NP$_{1,2,328-498}$ | 1,2 ——    328——498 | + | – |

[a] L cells (H-2$^k$) were transfected with the gene encoding the D$^b$ protein as well as the complete 1968 *NP* gene (1–498), and the truncated gene segments encoding residues 1–130 and 1,2,328–498 of the nucleoprotein.
[b] CTL lines specific for the influenza nucleoprotein were derived from influenza-infected C57BL/6 (H-2$^b$) and CBA (H-2$^k$) mice.
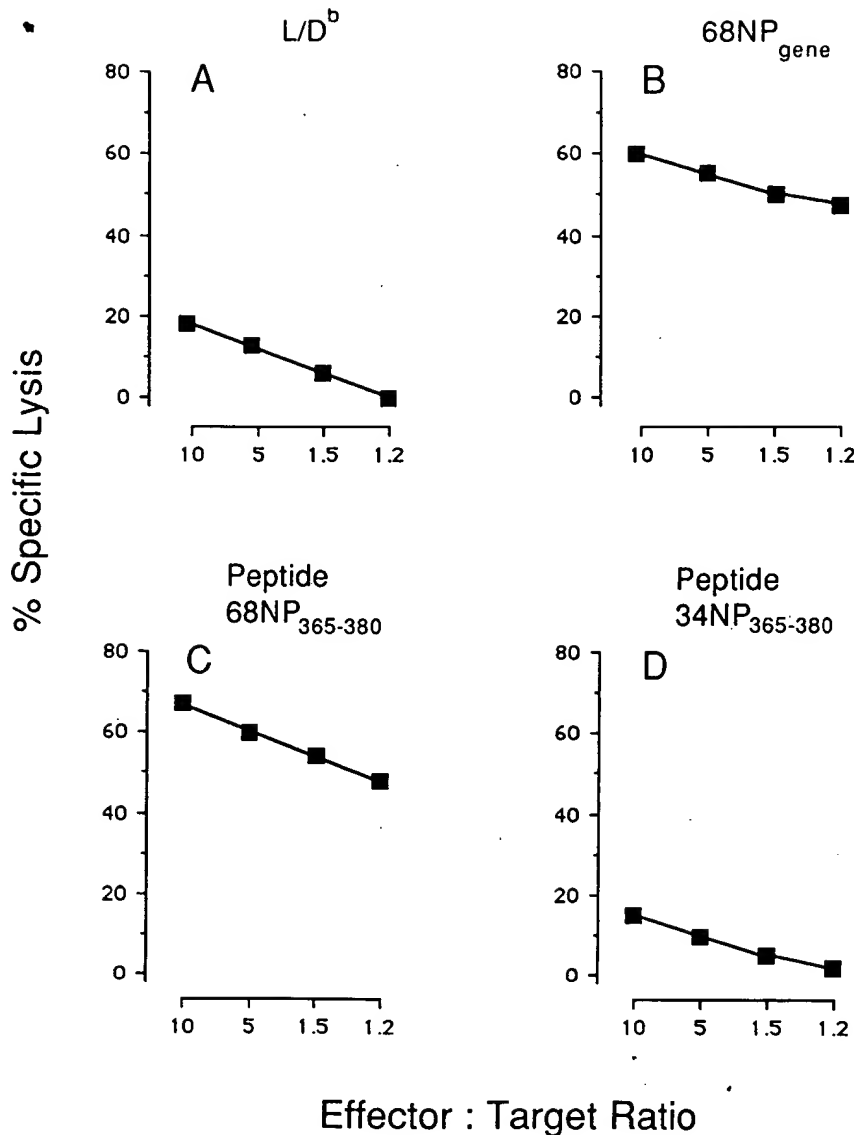Adapted from Townsend et al. (25).

**FIG. 4.** Recognition of peptides by a nucleoprotein specific cytotoxic T cell clone. The background lysis of L cell fibroblasts (L cells) transfected with the class I MHC gene $D^b$ by an influenza nucleoprotein (1968 NP) specific cytotoxic T cell clone is shown in **A**. This $D^b$ expressing cell line was used as a T cell target after cotransfecting with the gene for the 1968 nucleoprotein (**B**) or following incubation with peptides from the 1968 (**C**) and 1934 (**D**) sequences. (Adapted from Townsend et al., ref. 26.)

Effector : Target Ratio

synthetic peptide encompassing this segment sensitized cells for CTL lysis as effectively as the endogenously expressed *NP* gene.

There still remained a possibility that the requirement for antigen processing was a unique feature of the recognition of internal proteins such as NP. Maryanski et al. (27) addressed this question by demonstrating specific CTL recognition of a peptide derived from a glycoprotein target antigen—the HLA class I membrane protein expressed on a transfected mouse cell line. This result suggested that degradation of the endogenous gene product was likely to be a general requirement for class I-restricted T cell recognition.

Experiments using three forms of the same protein antigen, membrane bound, cytosolic, and secreted, confirmed the general necessity for processing. Influenza virus hemagglutinin, a membrane-bound glycoprotein, is recognized by a minor population of class I-restricted cytotoxic T cells from infected mice. Townsend and his colleagues (28) generated a cytosolic form of hemagglutinin

by genetically deleting the sequence encoding the amino terminal leader segment of the nascent protein, necessary for translocation across the endoplasmic reticulum. Consequently, the truncated hemagglutinin is confined exclusively to the cytosol. In a separate study, Braciale and co-workers (29) removed the transmembrane spanning sequence of the hemagglutinin while leaving the leader segment intact. In contrast to the wild-type or the leader-deleted protein, this form of hemagglutinin is efficiently secreted from cells as a consequence of deletion of the membrane anchor. The key to these manipulations was the demonstration that hemagglutinin specific, class I-restricted CTLs raised against virus-infected cells recognized transfected cells expressing the secreted and cytosolic forms of hemagglutinin just as well as they recognized the usually membrane-bound form. These results established that a processed form of the antigen and not the native molecule was the form relevant to class I-restricted T cell recognition. Moreover, the data suggested that all three forms of the protein had access to

the processing machanism and expression pathway necessary for class I-restricted antigen presentation.

## PEPTIDE BINDING STUDIES

### Class II Molecules Bind Peptides

While it was evident for some time that T cells recognized a combination of antigen and MHC products, it was not totally clear whether this combination represented a stable complex or whether the association between the MHC-encoded restriction element and antigen was transient and only stabilized by the presence of the T cell receptor. Early experiments by Rock and Benacerraf (30) on amino acid copolymers and by Werdelin (31) on haptenated polymers had demonstrated antigen competition by related antigens at the level of accessory cell presentation. Once it was established that peptides represented the actual form of antigen associated with the class II MHC molecule, investigators began to question whether these short, antigenic peptides could form stable and specific complexes with their restricting molecules.

Unanue and his colleagues were the first to successfully demonstrate a stable complex consisting of an immunogenic peptide and its purified Ia restricting element (32). To do this, they labeled the peptide with a fluorescent tag and then assessed its binding to detergent-solubilized Ia in a series of equilibrium dialysis experiments. The choice of peptide and Ia was dictated by their previous identification of the fragment 46 to 61 from hen egg white lysozyme ($HEL_{46-61}$) as the immunodominant determinant required for I-A$^k$-restricted T cell recognition. Specific binding of peptides to Ia was assessed as the fluorescence intensity associated with Ia. As shown schematically in Fig. 5, the labeled $HEL_{46-61}$ bound to the I-A$^k$ molecule but not to the product of the nonresponder allele, I-A$^d$.
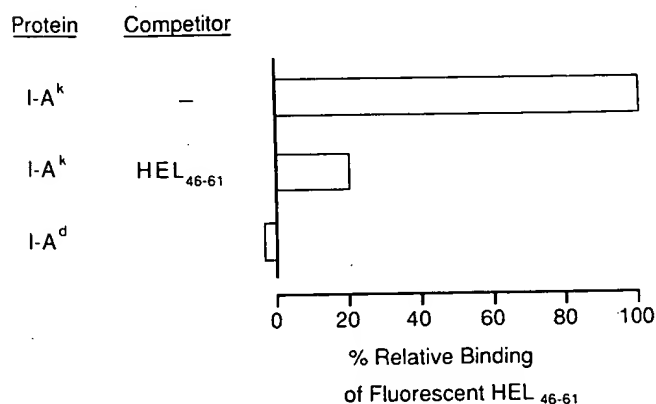
Excess, unlabeled $HEL_{46-61}$ could effectively compete with the fluorescently labeled $HEL_{46-61}$, indicating that this binding was peptide specific (Fig. 5). The binding of peptide to I-A$^k$ exhibited a dissociation constant ($K_d$) of approximately 2 μM. This was the first demonstration of haplotype specific Ia association for an immunogenic peptide.

### Ia Has a Single Peptide Binding Site

While these equilibrium dialysis experiments demonstrated peptide-Ia binding, it remained to be proved that such association had any relevance to T cell recognition. Babbitt et al. (33) were the first to provide evidence that this was the case. They began by demonstrating specific inhibition of the binding of solubilized I-A$^k$ and fluorescently labeled $HEL_{46-61}$ using a series of related and unrelated peptides. The abilities of this series of peptides to compete with fluorescent $HEL_{46-61}$ for binding to purified I-A$^k$, to inhibit activation of a specific T cell hybridoma, and to directly activate the T cell are compared in Table 11. Significantly, only the peptides that competed for Ia binding in solution were able to inhibit antigen presentation to the T cell hybridoma. This result provided strong evidence that the binding of an immunogenic peptide to its Ia-restricting element in detergent solution was related to its presentation to T cells. While others had shown antigen competition at the level of accessory cell binding, this was the first definitive proof that such competition experiments provided an accurate assessment of Ia–peptide association. Note that not all competing peptides are able to stimulate the specific T cell. $desTyr^{53}HEL_{50-61}$, for example, presumably binds to the same site on I-A$^k$ as $HEL_{46-61}$ but is not recognized by the T cell. Since $Gln_{49}Phe_{56}HEL_{49-61}$ which is the autologous mouse lysozyme sequence (Table 11) can inhibit both binding and presentation of $HEL_{46-61}$, the results also highlight the



Protein    Competitor

I-A$^k$    —

I-A$^k$    $HEL_{46-61}$

I-A$^d$

0   20   40   60   80   100

% Relative Binding
of Fluorescent HEL$_{46-61}$

**FIG. 5.** Binding of fluorescent hen egg lysozyme $HEL_{46-61}$ synthetic peptide to purified Ia. Binding of fluorescent labeled $HEL_{46-61}$ to detergent-solubilized I-A$^k$ and I-A$^d$ was measured as the fluorescence intensity associated with Ia following equilibrium dialysis. Binding of fluorescent $HEL_{46-61}$ to I-A$^k$ was also measured in the presence of 10-fold molar excess of unlabeled $HEL_{46-61}$. (Adapted from Babbitt et al., ref. 32.)

**TABLE 11.** The relevance of peptide–Ia binding in the $HEL_{46-61}/IA^k$ system

| Peptide | Ability to compete with Ia binding[a] | Ability to compete with activation[b] | Ability to stimulate $HEL_{46-61}$ T cells[c] |
|---|---|---|---|
| $HEL_{46-61}$ | Yes | — | Yes |
| $HEL_{49-61}$ | Yes | — | Yes |
| $Gln_{49}Phe_{56}HEL_{49-61}$ (=mouse) | Yes | Yes | No |
| $desTyr_{53}HEL_{50-61}$ | Yes | Yes | No |
| $HEL_{58-61}$ | No | No | No |
| $HEL_{53-57}$ | No | No | No |
| Fibrinopeptide B | No | No | No |

[a] Inhibition of the binding of fluorescent $HEL_{46-61}$ to purified I-A$^k$ in detergent solution.
[b] Antigen presentation to 3A9 T cell hybridoma by macrophages or planar lipid membranes.
[c] T cell hybridoma 3A9 as the indicator.
Adapted from Babbitt et al. (33).

inability of I-A$^k$ to descriminate between self and nonself in this instance. We return to this important point later.

Guillet et al. (34) decided to use peptide inhibition of T cell activation to assess the ability of *unrelated* as well as related peptides to bind to a given Ia molecule. They made use of T cell hybridomas specific for the sequence 15 to 26 of the bacteriophage λ repressor cI protein (cI$_{15-26}$), which is recognized in association with the I-A$^d$ molecule. A nonstimulatory, truncated form of this peptide, cI$_{12-24}$, could effectively inhibit the activation of the I-A$^d$-restricted T cell hybridoma 7B7.3 by cI$_{15-26}$ as shown in Table 12. In addition, two unrelated peptides, one from *Staphylococcus aureus* nuclease (Nase$_{61-80}$) and the other from ovalbumin (OVA$_{323-339}$) could also inhibit cI$_{15-26}$ specific T cell activation (Table 12) but failed to inhibit the I-E$^k$-restricted T cell hybridoma (8I) which is also specific for cI$_{15-26}$. Both Nase$_{61-80}$ and OVA$_{323-339}$ are themselves immunogenic in H-2$^d$ mice and restricted to I-A$^d$. Guillet and co-workers interpreted this competition between unrelated peptides as evidence for a single peptide binding site on the I-A$^d$ molecule. Peptide bound at this site could inhibit the subsequent association of any other I-A$^d$-restricted peptide whether the two were obviously related or not. In keeping with this proposal, an immunogenic peptide not restricted to I-A$^d$ should be unable to inhibit I-A$^d$ specific binding. Table 12 also shows that a second peptide from *S. aureus* nuclease, Nase$_{81-100}$, which is restricted to the I-E$^k$ molecule, failed to inhibit cI$_{15-26}$ recognition by the T cell hybridoma 7B7.3.

Meanwhile, Buus et al. (35) had extended the earlier equilibrium dialysis experiments to include the specific association of the immunodominant peptide from ovalbumin, OVA$_{323-339}$, to its restricting element, I-A$^d$. They then proceeded to examine the kinetics of peptide–Ia association and dissociation. To their surprise, they found that once the complex between Ia and peptide was formed, it proved to be remarkably stable. The overall equilibrium constant of approximately $2 \times 10^{-6}$ M was in fact due to a very slow off rate of the complex ($K_d \approx 3 \times 10^{-6}$ M$^{-1} \cdot$ sec$^{-1}$) and a rather slow rate of complex formation ($K_a \approx 1$ M$^{-1} \cdot$ sec$^{-1}$). The rate of dissociation was sufficiently slow that they were able to separate Ia-

bound and free peptide simply by using gel filtration chromatography. I-A$^d$-bound OVA$_{323-339}$ purified in this manner was capable of eliciting an ovalbumin specific T cell response when incorporated into supported planar membranes and was nearly 20,000 times more potent for this stimulation than free peptide mixed with I-A$^d$-containing planar membranes. Watts and McConnell (36) described virtually identical findings. Moreover, by using a sensitive fluorescent energy transfer system, they estimated that as few as 1,000 I-A$^d$-associated peptide molecules could effectively stimulate the response of a single ovalbumin specific T cell. All these results suggested that Ia had a single site capable of "trapping" peptide antigens in a stable, immunogenic form.

## Peptide Binding Site of Class I MHC Antigen

Biochemical data conclusively demonstrating a stable association between class I MHC proteins and peptide antigens have yet to be reported. However, analysis of the recently described crystal structure of the human class I histocompatibility antigen, HLA-A2, by Bjorkman et al. (37,38) has provided compelling evidence that class I MHC most likely binds peptide antigens in a manner analogous to that defined for the Ia molecule.

The key to this assertion is the presence of a prominent groove on the top of the HLA-A2 molecule. This groove consists of "walls" formed by two α helices, one from the α$_1$ domain of the HLA molecule and the other from the α$_2$ domain. The "floor" of the groove is formed by a series of β strands from both the α$_1$ and α$_2$ domains. This is shown schematically in Fig. 6. The overall structure consists of a cleft that is presumed to be the peptide binding site. The residues around this cleft are relatively polymorphic over a number of HLA sequences (Fig. 7). Residues on the inner "walls" and "floor" are the most polymorphic and it is thought that these contribute mainly to peptide binding. Residues on top of the two α helices that line the groove are also polymorphic but not to the same extent, and these regions are thought to make direct contact with the T cell receptor as noted in Fig. 6. During

**TABLE 12.** *Inhibition of* λ *repressor protein specific T cell hybridomas by various peptides*

| T cell hybridoma[a] | Specific inhibition (%) of T cell activation | | | |
|---|---|---|---|---|
| | cI$_{12-24}$ | Nase$_{61-80}$ | OVA$_{323-339}$ | Nase$_{81-100}$ |
| 7B7.3[b] | 65 | 75 | 75 | 1 |
| 8I[b] | ND[c] | 1 | 2 | ND |

[a] 7B7.3 is specific for λ repressor peptide cI$_{15-26}$ in the context of I-A$^d$, while 8I is specific for cI$_{15-26}$ in the context of I-E$^k$.
[b] T cell hybridomas 7B7.3 and 8I were cultured in the presence of cI$_{15-26}$ at a concentration which gave 50% of the maximal IL-2 release. Inhibition was assessed by adding excess amounts of the *nonstimulatory* peptides: λ repressor peptide cI$_{12-24}$, staphylococcal nuclease peptide Nase$_{61-80}$, staphylococcal nuclease peptide Nase$_{81-100}$, and ovalbumin peptide OVA$_{323-339}$.
[c] ND, not done.
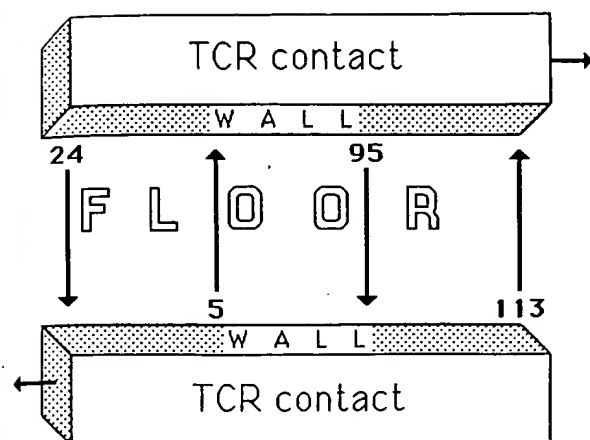Adapted from Guillet et al. (34).

**FIG. 6.** A highly schematized representation of the top of the HLA.A2 class I molecule based on the crystallographic data of Bjorkman et al. Portions of the $\alpha_1$ domain, amino acid residues 1 to 90, and the $\alpha_2$ domain, residues 91 to 180 only are included. The presumed peptide binding groove has a "floor" composed of antiparallel $\beta$ strands from both domains, and "walls" composed of the face of the two long $\alpha$ helices represented here as *boxes*, the upper one from the $\alpha_1$ domain, the lower one from the $\alpha_2$ domain. Antigenic peptide may contact the floor and walls of the groove, while the T cell receptor may contact the upper surface of the helices as indicated. (Adapted from Bjorkman et al., refs. 37 and 38.)

the course of the crystallographic analysis, it became obvious that this groove contained "electron-dense" material that was not part of the HLA sequence. This naturally led to the suggestion that the HLA antigen binding cleft was almost completely occupied by a mixture of peptide antigens. This was surprising since the HLA protein had undergone extensive purification, which apparently did not result in appreciable peptide dissociation. Consequently, the class I molecules would have peptide binding abilities analogous to those attributed to the detergent-solubilized form of Ia, notably a single binding site that forms a very stable association with peptide. This would be consistent with the strong sequence homology between both classes of MHC gene products which predicts closely related protein structures.

## Immune Responsiveness to Class II-Associated Antigens

The crystal structure, sequence homology, and peptide binding data suggest that MHC class I and class II glycoproteins function by binding peptides derived from intracellular processing and present them on the cell surface for T cell recognition. T cells specific for either class of molecules use the same receptor elements and the distinction between class I and class II antigen presentation is probably achieved by differential processing mechanisms as will be discussed later. The putative peptide binding site, derived from the crystal structure of human HLA.A2, would accommodate peptides of between 8 and

20 residues. Many class I and class II MHC-restricted T cell determinants of this length have been identified.

The crystallographic and peptide binding data also provide us with a clue as to how MHC polymorphism affects T cell function. Most of the known polymorphic residues are located on the top of the MHC protein around the putative peptide binding cleft. While some of the variant residues may directly affect T cell receptor contacts, the majority are situated partially or exclusively *within* the cleft itself and may be inaccessible to the T cell receptor. Thus allelic variation at these residues would be more likely to affect peptide binding. We have already discussed how allelic variation within the MHC genes controls the ability to mount an immune response to a given antigen. The MHC-linked nonresponder status of an individual is thought to result either from a failure to form an antigen–restriction element complex on the cell surface or from a failure of the T cell receptor repertoire to recognize such a complex (holes in the T cell repertoire). Clearly, the first possibility could come about as a consequence of binding to only selective peptides due to sequence variations within the MHC binding cleft. The ability of the MHC products to bind to given peptides would then determine the responsiveness of that particular MHC–antigen combination. The inability to bind would result in nonresponsiveness.

The results of one of the initial Ia–peptide binding experiments suggested that MHC-linked responsiveness versus nonresponsiveness was unlikely to be controlled exclusively at the level of MHC–peptide complex formation. As already shown in Table 11, Babbitt et al. (33) demonstrated that a mouse lysozyme peptide could ef-
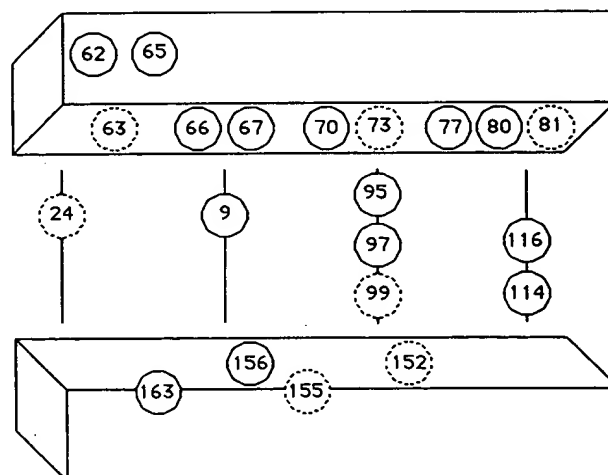


**FIG. 7.** The location of the most polymorphic residues in the $\alpha_1$ and $\alpha_2$ domains of the HLA class I molecule is indicated. Polymorphic positions are indicated by a *solid circle* for human sequences or a *dashed circle* for murine sequences. Note that many of the polymorphic residues are on the floor and walls of the presumed peptide binding groove. Residues on the $\alpha$ helices, depicted as *boxes*, which point up are shown in the middle of the top of the box. Residues 163 and 155 may point up toward the T cell receptor and in toward the peptide site. (Adapted from Bjorkman et al., refs. 37 and 38.)

fectively inhibit binding to the murine I-A$^k$ molecule. For this particular combination, Ia clearly fails to discriminate self from nonself. Mice that express I-A$^k$ do not respond to their own lysozyme presumably due to T cell tolerance, that is, a hole in the repertoire. However, it is difficult to assess the extent of immune regulation by Ia–peptide determinant selection based on just one example, particularly when this is a self-peptide. In an effort to better understand the capacity of Ia binding to control the T cell response, Buus et al. (39) decided to look at the relationship between T cell recognition and Ia association for a relatively large group of peptide–Ia combinations. In their experiments, they measured the association of 12 immunogenic peptides to each of four purified Ia molecules. This association was determined by direct binding to solubilized Ia and indirect "cold" peptide inhibition using four selected peptide–Ia combinations.

The results are shown schematically in Table 13 with the binding to designated restricting elements for each peptide enclosed within a box. Focusing attention only on the binding of each peptide to its designated restricting element reveals a specific interaction in each of the 12 instances. This suggests that specific Ia–peptide binding is necessary for a peptide to be an immunogen. Extending the analysis to include binding of each peptide to other Ia molecules shows that in many cases the peptides also bind to Ia molecules that do not serve as their MHC-restricting element. Consequently, while the ability of a peptide to bind to Ia may be a requisite for immunogenicity as described above, the binding to Ia may not in itself be sufficient to ensure this immmunogenicity. This is emphasized by the observation that the peptide cl$_{12-26}$ bound to I-E$^d$, which does not serve as a restriction element for it, better than to I-A$^d$ and I-E$^k$, which do serve as restricting elements for this peptide.

Buus et al. (39) concluded that their data supported the concept that both determinant binding by Ia as well as the shortcomings of T cell repertoire defined the immune response status to a given antigen. The observation that the *absence* of peptide–Ia binding was never seen for any

**TABLE 13.** *The capacity of purified Ia$^d$ and Ia$^k$ molecules to bind to various peptides*

| Peptide | Binding to Ia$^a$ | | | |
| --- | --- | --- | --- | --- |
| | I-A$^d$ | I-E$^d$ | I-A$^k$ | I-E$^k$ |
| Ovalbumin OVA$_{323-339}$ | [+ +] $^b$ | – | –/+ | – |
| Influenza hemagglutinin HA$_{103-142}$ | [+ +] | – | + + | – |
| Sperm whale myoglobin MYO$_{106-118}$ | [+ +] | – | + | – |
| λ repressor protein cl$_{12-26}$ | [+] | + + | – | [+] |
| Hen egg lysozyme HEL$_{46-61}$ | – | – | [+ +] | – |
| Hen egg lysozyme HEL$_{74-86}$ | + | +/– | [+] | +/– |
| Hen egg lysozyme HEL$_{81-96}$ | +/– | – | + | [+ +] |
| Influenza hemagglutinin HA$_{111-122}$ | – | [+ +] | – | +/– |
| Sperm whale myoglobin MYO$_{132-153}$ | – | [+ +] | – | + |
| Herpes simplex virus glycoprotein D HSV$_{8-23}$ | – | [+ +] | – | [+/–] |
| Pigeon cytochrome *c* pCytc$_{88-104}$ | +/– | – | +/– | [+ +] |
| Moth cytochrome *c* mCytc$_{88-103}$ | – | +/– | – | [+ +] |

$^a$ Peptide binding was assessed as direct binding of radiolabeled peptides to each of the purified and detergent-solubilized Ia molecules, and as "cold" peptide inhibition of each solubilized Ia molecule binding to a representative radiolabeled peptide.

$^b$ The known MHC restriction for each peptide is enclosed in a box.

Adapted from Buus et al. (39).

immunogenic peptide clearly favored the idea that the immune response is controlled by the ability of MHC proteins to form a stable association with a given peptide derived from intracellular processing. Stable peptide–MHC complexes must form prior to T cell recognition and the inability to form such complexes would result in nonresponsiveness. For example, $HEL_{46-61}$ fails to give a T cell response in $H-2^d$ mice because this peptide does not associate with either $I-A^d$ or $I-E^d$.

What determines the binding of peptide to MHC? Obviously the residues located within the MHC binding cleft play a decisive role in MHC–peptide interactions. This can be demonstrated by considering the differences between B10.A and B10.A(5R) in their immune response toward the pigeon cytochrome $c$ molecule. It was shown by Ronchese et al. (40) that allelic variation at a single residue (position 29) in the $I-E\beta$ chain controlled this response. When residue 29 is a valine, as is the case in B10.A mice, then pigeon cytochrome $c$ induces a vigorous T cell reaction. In contrast, B10.A(5R), which is a nonresponder for this protein, has a glutamic acid at residue 29 of $I-E\beta$. When Brown et al. (41) constructed a three-dimensional model of the class II MHC molecule based on the sequence homology between class I and class II MHC proteins, they found that residue 29 of $I-E\beta$ is located at the floor of their proposed peptide binding cleft. Consequently, allelic variation at this single residue situated within the proposed peptide binding cleft may determine whether the MHC allele can bind and successfully present pigeon cytochrome $c$ for T cell recognition.

The data on peptide–MHC binding provided in Table 13 also provide evidence that holes in the T cell repertoire sometimes contribute to MHC-linked nonresponsiveness. For example, λ repressor protein 12 to 26 binds strongly to $I-E^d$ but fails to elicit an $I-E^d$-restricted T cell response and, as already mentioned, mouse lysozyme can associate with $I-A^d$ but obviously does not generate a specific T cell response within mice. The concept to emerge from the combined experiments is that MHC proteins can bind to many different, but not to all, peptides. While this binding is necessary, it does not ultimately fully control the immune response, which rests at the level of T cell repertoire generation and selection. (As will be discussed later, receptor selection is itself under the control of the MHC-encoded class I and class II molecules.)

### Understanding *Ir* Gene Phenomena

The control of the immune system by *Ir* genes can now be explained in terms of the increasingly better characterized cell surface glycoproteins coded by the MHC. It is the function of these molecules to bind peptide fragments produced by intracellular degradation and to present them for T cell recognition. Failure to bind and to subsequently present a peptide results in selective unresponsiveness due to an apparent defect in antigen presentation. On the other hand, not all peptides presented by the MHC molecules elicit a T cell response for the simple reason that there exist limitations on the pool of

possible T cell receptors—the so-called holes in the T cell receptor repertoire. Whether this limitation in the T cell repertoire results exclusively from the deletion of self-reactive T cell clones or reflects fundamental constraints on the generation of T cell receptor diversity is unknown. Regardless of the identified limitations in the ability of MHC proteins to bind to certain peptide fragments, it remains a mystery how a given MHC product can still succeed in presenting such a vast array of peptides. For example, within the $H-2^b$ strain of mouse the T helper immune response to *all* soluble proteins is controlled by a single class II MHC product, $I-A^b$. It remains a challenge to elucidate the rules of MHC–peptide interactions. The recently described crystal structure of the MHC will no doubt play a large part in unraveling the puzzle of MHC restriction. Identifying the exact location of the residues involved in various MHC-associated functions will allow us to map the various sites within the MHC molecule and provide a structural basis to further characterize the trimolecular complex between MHC, antigen, and T cell receptor.

### ENDOGENOUS AND EXOGENOUS PATHWAYS OF ANTIGEN PRESENTATION

The two major classes of T cells, helpers and killers, recognize two different worlds of foreign protein antigens. As a broad generalization, class I-restricted CTLs recognize cell-associated antigens such as those on virus-infected cells or cells expressing foreign alloantigens. Class II-restricted T cells, on the other hand, recognize antigens that enter the system as soluble components. Essentially, the class I universe of peptides derives from endogenously synthesized proteins while the class II universe derives from proteins taken up by antigen-presenting cells from the external medium. Such a division makes great sense biologically since cells that harbor replicating intracellular parasites should be recognized and attacked by lytic cells. On the other hand, B cells, macrophages, or dendritic cells, which endocytose foreign antigens and which express class II glycoproteins, require positive signals from T cells to encourage them to make more antibody or to become activated.

This division of the antigenic world into killer and helper categories has been apparent for some time. However, it is only the recent demonstration that both antigenic worlds are reduced to peptide fragments that retain no obvious class I-ness or class II-ness that has brought the problem of how it is achieved into sharp focus. The problem is simple. When we introduce a soluble antigen such as lysozyme into the immune system, class II-restricted but not class I-restricted T cells are activated. The introduction of a live infectious virus will induce class I-restricted CTLs but a killed version of the same virus in general does not. Germain suggested that the division of the world into class I- and class II-restricted antigens depended on the independent sorting of class I and class II molecules within separate intracellular vesicles (42). He proposed that vesicles carrying class II molecules inter-
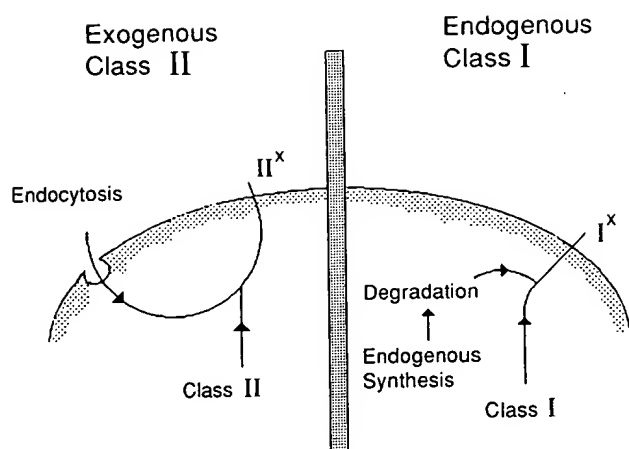
Exogenous
Class II

Endogenous
Class I



**FIG. 8.** Two possible pathways of antigen processing and presentation. On the **left** the exogenous or class II MHC-restricted pathway, on the **right** the endogenous or class I MHC-restricted pathway. Exogenous antigens that enter a cell by endocytosis, either receptor mediated or fluid phase, may be degraded in endocytic vesicles which eventually fuse with vesicles containing available class II molecules but no available class I molecules. The degradation of proteins made endogenously on free or membrane-bound ribosomes results in the peptides binding to class I molecules.

sect the endosomal cycle carrying the degraded form foreign proteins taken up from outside the cell while class I molecules are allowed to interact with an undefined pathway of degraded endogenously synthesized proteins (Fig. 8). The existence of this other pathway of antigen processing and presentation with class I had previously been postulated by Townsend et al. (25). For this explanation to be correct, it is important that the degradation of endogenous and exogenous proteins occurs separately.

Many of the salient features that distinguish class I-versus class II-restricted antigen presentation are illustrated by the work of Morrison and co-workers (43). They had established a number of cytotoxic T cell clones with specificity for the hemagluttinin (HA) of A/JAP/57 influenza virus. Some of the clones were classically class I, *H-2K* or *D* region restricted and some were of a minority

type and class II, *I* region restricted. (Note: The special biological significance of class II-restricted CTLs is not known.) Both class I- and class II-restricted CTLs could lyse target cells expressing the appropriate restriction element following infection by live A/JAP/57 virus. However, when UV-inactivated virus was used to pulse the target cells only the class II-restricted CTLs could recognize their determinant. Furthermore, when purified HA protein was added to the target cells only the class II-restricted CTLs caused lysis (Table 14). On the other hand, when target cells were infected with recombinant vaccinia virus carrying the influenza HA gene (but no HA protein), then the class I-restricted determinant formed readily on the cell surface but the class II-restricted target determinant did not (Table 15). These results suggested that the HA protein must be made inside the target cell in order to form the class I–peptide complex. Since both the class I- and class II-restricted T cells recognize peptide fragments of HA, this is clear evidence that endogenously synthesized proteins provide peptides for class I presentation while exogenously added proteins that are endocytosed provide peptides that associate with class II molecules. Both the class I-restricted and class II-restricted CTLs recognize targets that have been incubated in appropriately fragmented HA or the correct synthetic peptide.

While the experiments summarized above and the force of the biological argument are strong reasons to believe in the existence of two separate antigen presentation pathways, there may be some wrinkles in the system. For example, it has been proposed that the class I pathway of antigen presentation may accept only endogenously synthesized antigens (42). But when we realize that the same peptide of influenza HA can form a complex with a class I molecule whether the HA has been synthesized on membrane-bound ribosomes to go to the membrane or be secreted (29), or whether it is made on free ribosomes and released into the cytosol (28), then one is led to the belief that any protein microinjected into a cell would enter the same pathway. This is what viruses are able to do even following inactivation of the genome. Some viruses, such as Sendai, fuse with the external membrane while some, like influenza, require the lower pH of endosomes to fuse. The result of this fusion is the microin-

**TABLE 14.** *Class II- but not class I-restricted lysis induced by nonreplicating antigen*

| Cytotoxic clone | Restriction | Specific lysis (%) of targets | | | |
|---|---|---|---|---|---|
| | | Unrelated virus infection | A/JAP infected | UV-A/JAP | Purified HA |
| 35-6 | Class I | 7 | 39 | 9 | 9 |
| 36-1 | Class I | 2 | 43 | 2 | 2 |
| G1 | Class II | 11 | 47 | 52 | 43 |
| U-12 | Class II | 2 | 47 | 42 | 38 |

*Note:* H-2-restricted, A/JAP influenza specific CTL clones were assayed for lysis of target cells which express the appropriate class I and class II antigens in a 6-hr $^{51}$Cr release assay. Target cells were preincubated with infectious unrelated strain B influenza virus, infectious A/JAP virus, UV-inactivated A/JAP virus, or purified hemagglutinin (HA). Adapted from Morrison et al. (43).

**TABLE 15.** *Class I- but not class II-restricted lysis of target cells infected with vaccinia/influenza HA recombinant virus*

| Cytotoxic clone | Restriction | Specific lysis (%) of targets | | |
|---|---|---|---|---|
| | | Vaccinia[a] infected | VV/HA[b] infected | A/JAP infected |
| 14-1 | Class I | 1 | 49 | 69 |
| 14-7 | Class I | 0 | 77 | 68 |
| G1 | Class II | 3 | 4 | 68 |
| U-12 | Class II | 1 | 0 | 69 |

[a] Refers to wild-type vaccinia virus.
[b] Refers to vaccinia virus that carries the influenza virus hemagglutinin (*HA*) gene.
*Note:* CTL clones specific for the A/JAP influenza HA were assayed for lysis of target cells which express the appropriate class I and class II restriction element. Target cells were infected with infectious virus and assayed for lysis in a 6-hr $^{51}$Cr release assay.
Adapted from Morrison et al. (43).

jection of the genome plus associated nucleoproteins and polymerases into the cytoplasm. It seems unlikely that the cell would be able to distinguish these exogenous components from endogenously made viral components, and therefore their degradation products would enter the class I pathway. Indeed there is evidence that in some cases inactivated virion particles can create a class I-restricted determinant *in vitro* or can vaccinate class I-restricted CTLs *in vivo*. While it is true that UV-inactivated influenza virions do not create the class I restriction for CTLs when added to target cells (see Table 14), a recent publication indicates that when UV-inactivated virus is further treated so that fusion with and microinjection into the target cell occurs more efficiently then class I-restricted determinants are induced (44). Yewdell et al. found that heat-inactivated, UV-inactivated virions readily made L929 fibroblasts susceptible to influenza specific, class I-restricted CTLs. This was true even though UV-inactivated virions did not induce susceptibility (Table 16). The significant effect of heat inactivation at 55°C was to destroy the activity of the neuraminidase. The result of this is that microinjection occurs more efficiently. Inactivated virus did not induce susceptibility to CTLs specific for a nonstructural component of the virus (NS1), confirming that the genome had been inactivated. Surprisingly, this form of infection with inactivated virus also induced susceptibility to HA specific CTLs in addition to NP and PB1 specific CTLs. HA, an integral membrane component of the virus, is not microinjected into the cytoplasm but becomes a part of the cellular membrane following virus fusion.

In addition to microinjection, there may be another more physiologically relevant way in which exogenously introduced, nonreplicating antigens may enter the class I pathway. According to this notion, phagocytosis (as opposed to pinocytosis) of cellular debris by specialized antigen-presenting cells may lead into the class I pathway of antigen presentation. Class I-restricted CTL responses to minor histocompatibility antigens or haptens can be induced *in vivo* with cells that express the wrong (i.e., nonself) MHC (45). Since H-2-mismatched cells cannot present antigen to self-H-2-restricted T cells, the most

obvious way to explain this result is to suggest that the "exogenous" cells have been phagocytosed and digested and the peptide products presented with class I molecules.

According to recent experiments, there may be three modes by which antigen may enter a pathway of degradation leading to peptide presentation with class I molecules (Fig. 9). First, endogenously synthesized proteins, whether they are made on free (cytoplasmic) ribosomes or on membrane-bound ribosomes, can be degraded and presented with class I. Second, proteins injected into the cytoplasm, and perhaps even those that fuse into the membrane, enter the pathway. Finally, it is possible that protein components of phagocytosed, nucleated cells may also enter the class I pathway of presentation. For class II-restricted presentation, the original suggestion stands:

**TABLE 16.** *Noninfectious influenza virions can give rise to class I-restricted CTL determinants*

| L929 targets infected with | Specific lysis (%) with CTL specific for | | | |
|---|---|---|---|---|
| | HA | NP | PB1 | NS1 |
| Uninfected | 5 | 11 | 6 | 7 |
| Infectious PR8 | 69 | 81 | 81 | 71 |
| UV PR8 | 14 | 16 | 8 | 7 |
| UV, 55°C PR8 | 46 | 71 | 77 | 10 |

*Note:* CTL were generated from splenocytes of CBA (H-2$^k$) mice which had been primed *in vivo* with recombinant vaccinia virus carrying one influenza *PR8* gene, either for hemagglutinin (HA), nucleoprotein (NP), polymerase (PB1), or nonstructural 1 protein (NS1), and boosted *in vitro* with autologous PR8-infected cells. CTLs generated in this manner show exclusive specificity for the influenza virus gene product in the vaccinia recombinant used for *in vivo* priming. Target cells were L929 fibroblasts that express class I (H-2$^k$) antigens. PR8 virus used to infect target cells was either infectious, UV-irradiated to inactivate the viral genome, or UV-irradiated and heated at 55°C for 30 min to also inactivate the neuraminidase activity.
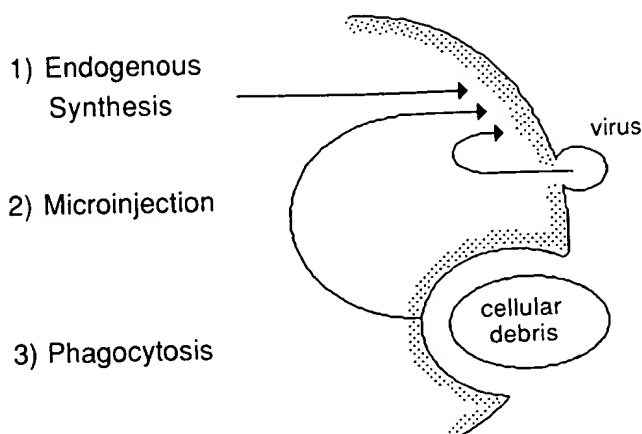Adapted from Yewdell et al. (44).

1) Endogenous
   Synthesis

2) Microinjection

3) Phagocytosis

virus

cellular
debris

**FIG. 9.** Three possible routes that may lead into the class I MHC-restricted pathway of antigen presentation: (1) endogenously synthesized self-proteins or viral proteins, (2) proteins that have been microinjected into the cytoplasm by fusion-competent, genome-inactivated viruses, and (3) specialized phagocytic cells in the lymphoid organs which may be able to traffic the degradation products of phagocytosed cellular debris to the class I pathway.

only exogenous antigens that are small enough to be endocytosed are presented with class II molecules.

## MHC ANTIGENS OF THE THYMUS SELECT THE T CELL REPERTOIRE

Experiments of researchers involving surgical thymectomy and experiments of nature giving rise to congenitally athymic animals established that the thymus is the site in which most or all T cells differentiate. Only a small fraction of the thymocytes produced in the thymus are allowed to exit to the periphery as functional T cells. The failure to make a productive rearrangement of the necessary TCR genetic elements may account for a great deal of the cell loss in the thymus. However, many immature, cortical thymocytes express an $\alpha,\beta$ TCR on the surface yet are never allowed to exit to the periphery. It seems likely that selective events focusing on the specificity of the TCR following its expression on the cell surface are responsible for the "passing" or "failing" of many thymocytes.

The years following the discovery that T cell responsiveness to antigen was restricted by the MHC antigens were dominated by discussions over whether a restricted T cell recognized the foreign X component and self-MHC somewhat independently (two-receptor models) or had specificity only for the complex of X plus MHC (one-receptor models). The experiments with $F_1$ animals and with MHC recombinant mice established that each immune T cell was restricted by only one MHC product. Furthermore, von Boehmer and Sprent were able to show with T cells from long-term double bone marrow chimeras that a T cell of H-2$^k$ genotype, for example, could be restricted to recognizing a foreign antigen in association with MHC class I or class II products of H-2$^d$. In these

"tetraparental" chimeras made by allowing a mixture of T cell depleted H-2$^k$ and H-2$^d$ bone marrow stem cells to reconstitute an irradiated $F_1$ host, it was demonstrated that H-2$^k$-derived T cells could collaborate with genetically allogeneic H-2$^d$ B cells as efficiently as with syngeneic, H-2$^k$ B cells. One interpretation of this finding proposed by von Boehmer and Sprent and by D. H. Katz was that during their maturation in the presence of allogeneic cells, T cells might "adapt" or "adaptively differentiate" to the foreign MHC.

Evidence that this indeed did occur was provided initially in the CTL system by Bevan (46) and Zinkernagel et al. (47) in 1977 and 1978. These workers showed that when H-2 heterozygous bone marrow cells were allowed to reconstitute the immune system of irradiated H-2 homozygous, parental mice, the restriction specificity of the $F_1$ T cells was dramatically skewed to recognizing antigen in association with host MHC alleles. An example of such ($F_1$ bone marrow → irradiated parent) chimera experiments is given in Table 17 for the CTL response to minor H antigens. $F_1$(H-2$^b$ × H-2$^d$) T cells from normal mice give roughly equal levels of b-restricted and d-restricted CTL activity in response to this antigen. In contrast, $F_1$ T cells from ($F_1$ → b) animals showed a strong preference for lysis of the antigenic H-2$^b$ targets while the CTL from ($F_1$ → d) animals were largely d restricted. In both cases the effector cells were shown to be derived from the $F_1$ marrow cells. Zinkernagel et al. (47) and Fink and Bevan (48) performed thymectomy–thymus grafting experiments to show the crucial role of the thymus in skewing the MHC restriction specificity of maturing T cells. In the experiments of Zinkernagel et al., $F_1$ mice were surgically thymectomized, lethally irradiated, and reconstituted with T cell depleted marrow also of $F_1$ origin. In the absence of a thymus, these mice would remain T cell deficient. The mice were then grafted with irradiated thymus lobes from one or the other parent. $F_1$ T cells matured in the thymus graft and their restriction specificity was ascertained 3 months later by immunizing the mice with vaccinia virus. In this situation in which the only H-2 homozygous component of the chimera is an irradiated thymus graft, it was again observed that the restriction specificity of the $F_1$ T cells was dramatically skewed to that of thymic MHC type.

These experiments were extended by a number of groups to include class II-restricted T cell responses (49,50). In fact, the self or thymic restriction of the T cell repertoire gained acceptance more rapidly in the case of class II-restricted T cells. However, it is now well accepted that both class I-restricted CTLs and class II-restricted helper T cells are selected by the MHC antigens expressed in the thymus such that only thymus-restricted T cells are allowed to emigrate. Furthermore, the cell type within the thymus responsible for this selection is (a) radiation resistant, (b) resistant to culture in the presence of 2-deoxyguanosine which eliminates most conventional antigen presenting cells, and (c) resident in the thymus since it remains with the thymus during serial transfer through an intermediate host. It thus appears likely that the critical cell type is a thymic epithelial cell and not an immigrant, bone-marrow-derived cell (51,52).

**TABLE 17.** *MHC molecules of the irradiated host determine the restriction specificity of T cells in an ($F_1 \rightarrow$ parent) chimera*

| Responding T cells | Immunogen | Specific lysis (%) of targets | | |
|---|---|---|---|---|
| | | H-2$^b$ + ag | H-2$^d$ + ag | F$_1$ |
| 1. Normal (Balb/c × Balb.B)F$_1$ | F$_1$ + minor H | 55 | 68 | 1 |
| 2. (F$_1$ → Balb/c) chimera | F$_1$ + minor H | 5 | 81 | 2 |
| 3. (F$_1$ → Balb.B) chimera | F$_1$ + minor H | 61 | 9 | 0 |

*Note:* Balb/c (H-2$^d$) or Balb.B (H-2$^b$) mice were lethally irradiated and reconstituted with (Balb/c × Balb.B)F$_1$ T-cell-depleted bone marrow cells. Eight weeks later these (F$_1$ → parent) chimeras and normal F$_1$ mice were immunized *in vivo* and *in vitro* with minor histocompatibility antigen disparate (B10.D2 × B10)F$_1$ cells. CTL activity was assayed on B10(H-2$^b$) targets, B10.D2(H-2$^d$) targets, and (Balb/c × Balb.B)F$_1$ targets.

Adapted from Bevan (46).

## Interpreting Thymic Selection

The results from the (F$_1$ → parent) chimeras and the subsequent thymus grafting experiments showed that MHC restriction specificity is imposed on the maturing T cell pool in the thymus. What this means is that a T cell that scores later in the periphery as specific for self-D$^b$ plus a nucleoprotein peptide or for I-A$^k$ plus a KLH fragment was actually selected by self-D$^b$ or I-A$^k$ molecules in the thymus *in the absence of the foreign peptide*. Thymus selection of MHC restriction specificity obviously hints at the independent recognition of self-MHC molecules (i.e., it occurs in the apparent absence of foreign antigen). [In fact, the discovery was sufficient to convert Zinkernagel to a belief in the two-receptor model T cell specificity (47).] The more recent data that have accumulated showing that complexes of antigenic peptide and Ia form, that MHC molecules possess a groove which holds peptide securely, as well as the data on the TCR itself, are all strong arguments for the idea that restricted T cells recognize antigen–MHC in much the same way as antibody binds its ligand. How then are we to allow thymic selection of a T cell repertoire which will have reactivity preferentially to self-MHC plus foreign peptides? It seems we have two choices: (a) the older "affinity argument," which states that low-affinity TCR–MHC interactions occur in the thymus and are enough to give positive selection—but these low-affinity interactions cannot be measured in mature T cells—or (b) the recent proposal by Marrack and Kappler that thymic epithelial MHC molecules may be modified in a unique way to allow selection to occur (53).

## The Affinity Model

According to this type of model, the cortical thymus epithelial cells are responsible for allowing the survival of newly emerged α,β TCR-bearing thymocytes if these receptors have a certain minimum affinity for the MHC antigens. Very-low-affinity interactions of the TCR with epithelial MHC molecules allow positive selection to occur. At a later stage, possibly mediated most efficiently by macrophages or dendritic cells in the thymus, any self-MHC-selected T cell from the cortex will be screened for tolerance induction. In this test, TCR with affinity for MHC molecules above a certain threshold will be eliminated ("clonal deletion"). The key element to this model of thymic selection of self-MHC restriction preference is that some of the cells positively selected by thymic epithelial MHC molecules survive the tolerance induction step. That is, T cells that express self-reactive receptors within a *window of affinities* survive. These survivors become the peripheral pool of T cells which are unable to respond to self-MHC molecules and self-peptides (because of the tolerance step). They are able to respond to a new complex of self-MHC plus appropriate foreign peptide when the peptide increases the overall affinity of the complex for the TCR.

There is no direct evidence to support this model. For example, it is not the case that low-affinity interactions are favored in the thymus because cortical thymocytes have a high surface density of the TCR, or cortical epithelial cells have high class I or class II expression. Rather, the cortical thymocytes that we observe have a low copy number of surface TCR, and class I expression is certainly not high on the epithelium.

## The Modified MHC Model

Recent results from X-ray crystallography and peptide binding have led to the notion that the majority of surface MHC molecules are always occupied by peptides. Most of the resident peptides we expect to be self-peptides. The suggestion has been made recently that the MHC molecules on the epithelial cells in the thymic cortex which select the repertoire may be modified in some absolutely unique way (53). For example, there may be thymus epithelium specific peptides produced which have affinity for the peptide binding site on the MHC molecules. The maturing T cell population, it is postulated, will be selected for survival on the basis of the affinity of their receptor for these self-MHC plus unique peptide complexes. Tolerance induction will *not* include these "selecting" peptides, which by definition would only be found in the thymus cortex. The cells that pass positive and negative selection steps will go to the periphery and

respond to foreign peptide–MHC complexes which mimic these selecting peptide–MHC complexes. Again, there is no direct evidence to support this model. It does predict that *every* mature T cell in the periphery could mount a strong autoimmune response to thymus epithelial cells. Clearly this is a potentially very dangerous situation.

## ALLOREACTIVITY

Alloreactivity or alloaggression refers to the finding that a surprisingly large fraction of T cells from an unimmunized animal responds to allogeneic stimulator cells. Only allogeneic differences coded by the MHC are able to stimulate such a large response; genetic differences in other surface molecules do not. [The mouse is an exception here, since the *Mls* locus also encodes highly stimulatory antigen(s)!] This phenomenon was one of the early links between T cell specificity and the MHC. As far as we know, it has no physiological significance since the only natural exposure to allogeneic cells is during pregnancy, and nonviviparous animals such as birds display the phenomenon of alloreactivity and genetically homozygous strains of mice breed perfectly well. However, understanding alloreactivity is an intellectual challenge and may shed light on the physiological responsiveness to self-MHC molecules complexed with foreign peptide.

### The Phenomenon of Alloreactivity

As few as 50 to 100 leukocytes from an adult chicken deposited on the chorioallantoic membrane of 10- to 12-day-old allogeneic chick embryos were able to produce a local graft-versus-host reaction visualized as a pock. The high frequency of responsiveness correlated with MHC differences (54,55). Alloreactivity could also be measured *in vitro* by coculturing lymphoid cells from two strains (mixed lymphocyte culture, MLC) and assaying the degree of proliferation 3 to 5 days later. Again, only MHC-encoded differences gave measurable responses.

Quantitating the level of alloreactivity by calculating the fraction of normal lymphocytes from an MHC$^a$ animal

that can react to MHC$^b$ stimulator cells has been done *in vivo* and *in vitro*. Atkins and Ford (56) determined what fraction of rat T cells were specifically recruited into the spleen of an irradiated, allogeneic rat by comparing the recovery of intravenously injected cells with that of control, unresponsive T cells (Table 18). Potentially reactive cells were prelabeled with $^3$H-uridine and the control, nonreactive population was labeled with $^{14}$C-uridine (in some experiments the labels were reversed). The control population was either genetically unresponsive, had been neonatally tolerized to B with antigens or had been depleted of B reactive cells by "filtration" through an (A × B) F$_1$ host. By estimating the $^3$H/$^{14}$C ratios in spleen and thoracic duct lymph at early time points after intravenous injection, they were able to estimate the fraction of normal strain A cells which can recognize strain B antigens. This approach suggested that about 6 to 12% of T cells score as alloreactive. Actually, it seemed that about 12% of cells were sequestered in the central lymphoid organs and about half of these were subsequently activated to divide.

*In vitro* estimates of the frequency of alloreactive cells have been done by coculturing responder and stimulator cells together in MLC under limiting dilution condition. In these types of experiments replicate 0.2-ml wells are set up with graded, low numbers of responder lymphocytes, together with an excess of irradiated stimulator spleen cells and medium containing a supply of lymphokines. The response of each well may be assayed 3 to 5 days later for proliferating T cells by $^3$H-thymidine incorporation. Alternatively, specific cytotoxic T cell development may be assessed by adding $^{51}$Cr-target cells to each well. If a single responder cell type is limiting the response, the frequency of positive wells will follow the Poisson distribution. Figure 10 gives an example of results from this type of experiment in which 100 to 1,000 C57BL/6 (H-2$^b$) lymphocytes were cultured with H-2$^d$ stimulator cells and the frequency of measurable proliferating and cytotoxic T cell precursors calculated. These experiments have yielded minimum frequency estimates of about 1% (57,58).

In the case of the response to conventional foreign antigen, CD8$^+$ cytotoxic T cells recognize a degraded peptide in association with self-class I glycoproteins, while CD4$^+$ T cells recognize peptide plus self-class II glyco-

**TABLE 18.** *Measurement of alloreactivity by a systemic graft-versus-host reaction*

| $^3$H-labeled reactive population | $^{14}$C-labeled nonreactive population | $^3$H/$^{14}$C ratios | | |
|---|---|---|---|---|
| | | Original inoculum | Spleen | TDL |
| A | (A × B)F$_1$ | 1.00 | 1.22 | 0.87 |
| A | Filtered A$_{-B}$ | 1.00 | 1.21 | 0.89 |
| A | A neonatally tolerant to B | 1.00 | 1.18 | 0.92 |

*Note:* Thoracic duct lymphocytes (TDLs) from a normal strain A rat that can react to strain B alloantigens and nonreactive TDLs from three sources were labeled *in vitro* with $^3$H- or $^{14}$C-uridine, mixed and injected i.v. into irradiated (A × B)F$_1$ hosts. The ratio of labels in the spleen, lymph nodes, and thoracic duct lymph were measured by scintillation counting at 24 hr. Taking into account the percentage of total radioactivity recovered in these organs, the authors calculated that the anti-B reactive cells carry approximately 12% of the radioactive label in the starting population.
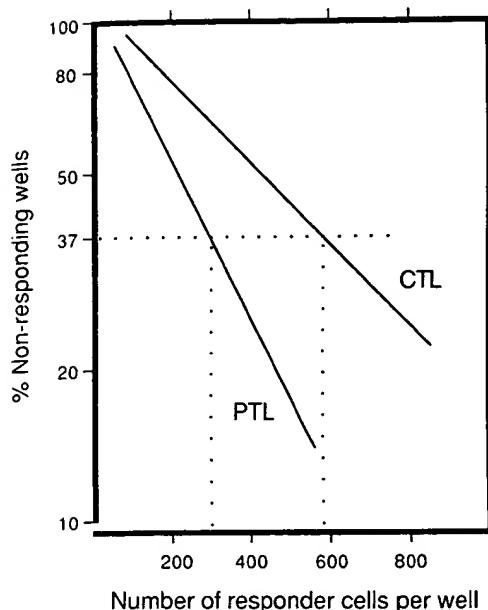
Adapted from Atkins and Ford (56).

**FIG. 10.** Determination of the frequency of alloreactive T cells in a limiting dilution assay. Responding H-2$^b$ spleen cells were cultured at 200 to 1,000 cells per well with an excess of irradiated stimulator cells of H-2$^d$ origin and medium containing lymphokines. Microcultures were assayed for $^3$H-thymidine incorporation (PTL) or for H-2$^d$ specific target cell lysis (CTL). The number of responder cells per well required to give 37% negative wells gives the precursor frequency of alloreactive T cells. In this case, PTL precursor frequency is 1/270 and CTL precursor frequency is 1/540 spleen cells. (Adapted from Lindahl and Wilson, ref. 57, and Ryser and MacDonald, ref. 58.)

into CD8$^+$CD4$^-$ and CD4$^+$CD8$^-$ subpopulations. The CD8$^+$ T cells reacted preferentially to cells expressing a class I mutation, while the CD4$^+$ cells responded to stimulators expressing a class II mutation (59).

These experiments also illustrate another fascinating feature of alloreactivity, namely, that an apparently very subtle change in the K$^b$ or the IA$^b$ molecule can be sufficient to activate a large fraction of T cells. These and other strongly stimulatory mutants differ from the wild type in only 1 to 3 amino acid residues and are usually serologically indistinguishable from the wild-type molecule.

## Alloreactive Cells Are Within the Same Pool as Self-Restricted T Cells and Use the Same Receptor

The influential Jerne hypothesis put forward in 1971 proposed that alloreactive T cells were a separate pool from those responding to conventional antigens (60). This was an attempt to explain the fact that the MHC coded both for Ir genes and for the strongly stimulating alloantigens. Jerne proposed that the T cell receptor repertoire had been selected during evolution to bind MHC molecules. Thymocytes expressing germline T cell receptors coding for allo-MHC were postulated to pass through the thymus to the periphery unhindered and unchanged (alloreactive T cells). Thymocytes expressing anti-self-MHC receptors were thought to be held up in the thymus and allowed to exit to the periphery only after their receptor accumulated mutations such that anti-self-MHC reactivity was lost. These mutants, derived from cells originally selected as anti-self, were proposed to be the cells responsive to conventional antigens and to express the Ir-gene-controlled phenomena.

Because the response to alloantigens is so exceptionally high, there have been repeated suggestions that a receptor other than the one used to react to conventional foreign antigen is used to recognize allo-MHC. The latest, and perhaps the last, of these is the "one and a half receptor" model (61). According to this suggestion, the γ or δ chain may be involved in allorecognition by a T cell that bears

protein. Predictably in the case of the alloreactive response, CD8$^+$ T cells respond preferentially to class I alloantigens and CD4$^+$ T cells to foreign class II antigens. A very clear demonstration of this is provided by the recent work of Sprent and Schaeffer (Table 19). Lymph node cells from normal C57BL/6 (H-2$^b$) mice were separated by a combination of antibody plus complement lysis followed by adherence to antibody-coated plates

**TABLE 19.** Proliferative response of T cell subsets to mutant class I or class II differences

| B6 responder T cells | $^3$H-thymidine incorporation ($\times$ 10$^{-3}$) in the presence of stimulator cells | | | |
|---|---|---|---|---|
| | B6 | (B6 × bm1) | (B6 × bm12) | (B6 × CBA) |
| CD4$^+$CD8$^-$ | 2.1 | 2.5 | 16 | 19 |
| CD8$^+$CD4$^-$ | 1.2 | 26 | 1.4 | 25 |

Note: Lymph node T cells from normal B6 mice were separated into two subsets by a combination of lysis with antibody and complement followed by positive selection on panning dishes coated with anti-CD4 or anti-CD8 antibody. The purified subsets showed less than 1% cross-contamination. MLCs were set up for 4 days in the presence of irradiated spleen cells that were syngeneic B6, expressing a K$^b$, class I mutation (B6 × bm1)F$_1$, expressing an I-A$^b$ class II mutation (B6 × bm12)F$_1$, or fully H-2 different (B6 × CBA)F$_1$.
Adapted from Sprent and Schaeffer (59).

**TABLE 20.** *Nonrandom pattern of alloreactivity displayed by a panel of self-MHC-restricted murine T cell clones*

| Conventional antigen | Number of clones | Number of cross-reactive clones for H-2 haplotypes tested for alloreactivity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | d | p | a | r | v | u | f | t4 | i5 |
| GAT | 16 | 3 | 4 | 2 | 3 | 3 | 7 | 3 | 4 | 1 |
| Sheep insulin | 13 | 0 | 4 | 5 | 0 | 0 | 8 | 0 | 1 | 3 |
| Pigeon cytochrome c | 9 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 5 | 4 |

*Note:* A large number of T cell clones were isolated by limiting dilution from B10.A(H-2$^a$) mice immunized with GAT, sheep insulin, or pigeon cytochrome c. These self-MHC-restricted T cells were tested for cross-reactivity on a panel of nine different allogeneic haplotypes.
Adapted from Ashwell et al. (62).

in addition a functional α,β receptor for conventional antigen.

No support has emerged for these "separate cell populations" or "separate receptor" views on the nature of alloreactivity. Instead, the following types of evidence suggest strongly that the pool of alloreactive T cells and self-MHC-restricted T cells is one and the same and that the specificity of both reactions lies in the same α,β TCR.

1. Dual-reactive T cells with specificity for a foreign MHC and for self-MHC plus a conventional, foreign antigen have been observed in numerous instances. Antigen-selected bulk T cell populations as well as T cell clones show this type of cross-reactivity.
2. In one extensive analysis of this cross-reactivity, Ashwell et al. (62) prepared a large number of T cell clones specific for self-class II plus foreign antigen and found that the majority cross-reacted with at least one member of a panel of nine allo-class II-expressing cells. Importantly, the pattern of cross-reactive alloreactivity was reproducibly tied to the self-MHC-restricted specificity of the clones, suggesting the involvement of the same receptor (Table 20).
3. In a case in which a monoclonal anti-idiotypic antibody against the α,β TCR of a dual-reactive T cell clone is available, the monoclonal antibody inhibits both forms of recognition (63).
4. With very rare exceptions, peripheral T cells that express a functional α,β TCR complex do not have a productively rearranged γ or δ gene. Furthermore, allelic exclusion applies to the α and β locus such that in any mature T cell only one locus is functionally rearranged.

## Current Models of Alloreactivity

### The Alloreactive Response Is to Peptide–MHC Complexes

In 1977 Matzinger and Bevan proposed an entirely new type of model to explain the high frequency of T cells that can respond to cells expressing a foreign MHC antigen (64). The hypothesis was based on the then controversial notion that T cells had one receptor with specificity for the complex of self-MHC and foreign antigen and that there could be no independent recognition of MHC alone or of X alone. This implied that recognition of all self-, non-MHC-coded components was dependent on the MHC and that tolerance to every component would be MHC restricted. For example, an H-2$^a$ mouse would be tolerant to the complex of mouse (self) serum albumin plus H-2$^a$ but would not be tolerant to mouse serum albumin plus H-2$^b$. Thus changing one MHC locus had the effect of creating a whole new universe of X–MHC complexes.

This hypothesis was written before one knew of the peptide binding site on the MHC molecule, or that class I molecules could transport any internal peptide to the cell surface. But the implications are the same as those we now glean from the structural work on the HLA-A2 class I molecule. Because of the analysis by Bjorkman et al. (37,38), the peptide–MHC complex view of alloreactivity is enjoying a revival. The salient features of this recent work are as follows:

1. The papain-cleaved HLA-A2 molecule apparently held peptide(s) in the groove between the two α helices during the entire process of purification and crystalization. It cannot be determined whether this electron-dense material (which is not part of the HLA-A2 primary sequence) is composed of one or a few species or of a multitude of species. Finding this peptide passenger fits very nicely with the notion of a slow off rate for peptide–class II complexes noted by Buus et al. (35). Perhaps the most important implication for alloreactivity is that MHC glycoproteins are never unoccupied, meaning that multiple peptide–MHC complexes may in fact normally exist on the cell surface.
2. The crystallographers were able to locate the polymorphic and variant residues of class I on the structure. One of the conclusions from this analysis is that many residues that affect CTL recognition of the molecule are clustered on the top of the molecule in the groove identified as the peptide binding site in a location where they may be inaccessible to the TCR. Thus their effect on alloreactive CTL recognition may be mediated via changing the spectrum of bound peptides.

In order to explore this suggestion further, we have drawn a highly simplified version of the T cell view of an

empty (i.e., peptide minus) HLA-A2 molecule (Fig. 6). The peptide binding groove consists of a "floor" of four β strands, the two on the left from the $\alpha_1$ domain, the two on the right from the $\alpha_2$ domain, and "walls" composed of the inwardly facing sides of the two α helices, the helix from the $\alpha_1$ domain on top. Residues that may make direct contact with the TCR are on the top of the α helices. What Bjorkman and colleagues noted was that the majority of the most polymorphic residues were located on the floor and walls of the peptide binding site where they would be more able to influence peptide binding than to have a direct affect on TCR binding. The location of these polymorphic sites is shown schematically in Fig. 7.

A direct test of the idea that the bulk of alloreactive T cells recognize peptide plus allo-MHC is to ask whether "pure" MHC class II molecules can be recognized. This type of experiment has been reported by Coeshott et al. (65). The class II glycoproteins $IA^d$ and $IE^d$ were purified by affinity chromatography from detergent lysates of a B cell lymphoma, incorporated into liposomes, and then bound to the surface of glass beads. In this form they were able to stimulate the release of IL-2 from the majority of $IA^d$ or $IE^d$ allospecific T hybridoma lines. The conclusion from this study was that alloreactive T cells do not need to corecognize a bound peptide in association with the class II molecule. However, now that we know that peptides can remain bound to MHC molecules during many days of purification, this initial conclusion is no longer valid. It seems likely that the purified Ia was in fact a mixture of peptide–Ia complexes. What one needs in order to improve on the experiment is a way to flush out the passenger peptide while retaining the basic structure of the molecule.

### Alloreactive T Cells Recognize MHC Antigen Alone

The alternative to believing that alloreactive T cells consist of a collection of distinct T cells each of which is specific for an allogeneic MHC molecule and one of the myriad of peptides conjugated to it is to believe that the interaction with the allogeneic stimulator cell is mainly with determinants on the MHC molecule itself (63,66). In the physiological situation, when, for example, a CTL responds to virus-infected autologous cells, the density of any one particular antigenic peptide–class I complex on the infected cell may be expected to be very low. Since class I (or class II) molecules themselves do not distinguish self from foreign peptides, the number of peptides competing for the site on the MHC molecule is enormous. Peptides will vary in their affinity for a particular allele of class I but one can suppose that only one in $10^3$ to $10^5$ copies of the surface class I may be complexed with any particular peptide. The self-MHC-restricted T cell has to have the ability to bind specifically to this rare complex determinant. Thus it may be that the response to a conventional, MHC-restricted antigen is mediated by T cells with high-affinity receptors for complex peptide–MHC determinants expressed at low density on the stimulator cell.

When the T cell receptor repertoire of one animal (which has probably been shaped by evolution and by thymus selection to focus on MHC antigens) is confronted with cells expressing an allogeneic MHC molecule, the situation differs from the one normally presented by conventional MHC-restricted antigens. In the case of allogeneic MHC there are some foreign determinants expressed on 100% of the molecule. The determinants recognized by monoclonal alloantibodies, for example, are present on all copies of the MHC molecule, since one antibody can precipitate all the molecules. Such allodeterminants are obviously expressed at a much higher density on the cell surface than are the self-MHC plus X determinants. In this case, T cells that have TCR with a low affinity for an allodeterminant on the MHC molecule will be able to respond. This view of the nature of alloreactivity predicts that if one could engineer a soluble TCR from an alloreactive T cell clone it would behave like a low-affinity alloantibody specific for an MHC antigen; that is, it would have specificity for most of the copies of the target MHC molecule on the cell surface. The peptide view of alloreactivity, on the other hand, predicts that an alloreactive TCR would have significant binding affinity for only a tiny fraction of the MHC–peptide complexes on a cell. Thus a soluble TCR from an alloreactive T cell would behave like an MHC-restricted antibody. It should be remembered that these very different explanations of the phenomenon of alloreactivity are not mutually exclusive and both may apply in different situations.

## HLA AND DISEASE

Unraveling the immunological role of the class I and class II MHC molecules represents a major achievement. That evolution has created these molecules with "jaws" that capture peptide fragments of diverse compositions, hold them almost irreversibly, and bring them out to the cell surface for perusal by a T helper cell population (class II) or a CTL population (class I) is a dazzling scenario. While there remain many things we have yet to understand about the system, "fundamental immunologists" can feel pride and pleasure in this biological masterpiece. But unless this warm feeling becomes too smug, we are forced to confront the issue of how susceptibility to many human diseases is linked to particular *HLA* alleles. As we shall see *none* of the associations between HLA and disease is understood. They all remain a mystery!

The human class I MHC antigens (HLA-A, B, and C) and class II MHC antigens (HLA-DR, DQ, and DP) function to restrict the activities of CTLs and helper cells in the same way we have outlined for other species. For example, it is known that (a) human peripheral T cells display the phenomenon of alloreactivity to cells bearing foreign HLA molecules—$CD8^+$ T cells respond well to HLA-A, B, or C differences and $CD4^+$ cells to HLA-D-encoded differences; (b) proliferative T cell responses to antigens on common human pathogens are restricted by HLA-D molecules and are specific for degraded forms of the antigen; and (c) human CTLs recognize peptide frag-

ments of viral antigens in association with self-class I HLA molecules.

A remarkable example of how the HLA class I allele determines the specificity of a CTL response has been provided by McMichael et al. (67). In studying the response to influenza A virus, they noted that the bulk of the cross-reactive CTL response in one donor was specific for the influenza nucleoprotein peptide $NP_{335-349}$ in association with HLA-B37. Screening 14 individuals of various HLA types, all of whom could kill influenza-virus-infected autologous cells, revealed that HLA-B37 seemed to be the only determining factor in whether $NP_{335-349}$ was the major target peptide. Thus four individuals possessed HLA-B37 and lysed autologous targets coated with peptide while 10 HLA-B37-negative individuals did not recognize this peptide.

With this reassurance that HLA molecules control the specificity of T cell responsiveness by selecting which peptides are presented at the cell surface, we now consider the disease associations.

## Disease Associations

Whether a certain disease condition is more (or less) likely to occur in a person carrying a particular HLA marker than in an individual who does not express the marker has been ascertained in a large number of studies. As it has turned out, many of the tentative associations did not stand the test of time. Some of the clearest examples, which have been confirmed by many independent

studies, are listed in Table 21. The difficulty in associating a disease with an HLA marker lies mainly in focusing on the true, causative HLA molecule. The main reason for this is that until recently HLA typing has been done largely with serological reagents (human alloantisera or murine monoclonal antibodies) which do not uniquely identify any particular allelic product, and which fail completely to recognize some allelic differences. HLA serological typing is being expanded by T cell typing tests such as primed lymphocyte typing (PLT), and more recently by restriction fragment length polymorphism (RFLP) analysis or by direct gene sequencing. Some diseases originally thought to be HLA class I-associated actually turned out to be more tightly associated with class II molecules of the HLA-D region. Two of the human class II molecules HLA-DR and HLA-DQ are closely linked and markers at the two loci exist in strong linkage disequilibrium. Diseases said to be "DR linked" may in fact show a much tighter correlation with a DQ-encoded αβ heterodimer.

The strength of an association is commonly expressed by the term "relative risk." The relative risk indicates how many times more frequently the disease occurs in individuals who express a certain HLA marker than in individuals who do not express the marker. A relative risk greater than 1 indicates a positive correlation of the disease with the marker, and the greater the value the stronger the association. The means of calculating the relative risk from a population study is shown in Table 22. In addition to population studies, for some of the diseases with familial tendencies, HLA associations have been confirmed by family studies.

**TABLE 21.** *Some significant HLA and disease associations*

| Disease | Associated HLA allele | Relative risk |
|---|---|---|
| Idiopathic hemochromatosis | A3 | 6.7 |
| Pemphigus vulgaris | A26 | 4.8 |
| Behçet's disease | B5 | 3.8 |
| Myasthenia gravis | B8 | 3.3 |
| Systemic lupus | B8 | 2.7 |
| Ankylosing spondylitis (Caucasian) | B27 | 69.1 |
| Ankylosing spondylitis (Oriental) | B27 | 207.9 |
| Reiter's disease | B27 | 37.1 |
| Acute anterior uveitis | B27 | 8.2 |
| Juvenile rheumatoid arthritis | B27 | 3.9 |
| Psoriasis vulgaris | B17 | 5.3 |
| Psoriasis vulgaris | Cw6 | 7.5 |
| Celiac disease | DR3 | 11.6 |
| Goodpasture's syndrome | DR2 | 13.8 |
| Juvenile diabetes mellitus | DR3 | 3.3 |
| Juvenile diabetes mellitus | DR4 | 3.6 |
| Rheumatoid arthritis | DR4 | 3.8 |
| Juvenile rheumatoid arthritis | DR5 | 3.9 |
| Narcolepsy | DR2 | 129.8 |
| Pemphigus vulgaris | DR4 | 14.6 |
| Dermatitis herpetiformis | DR3 | 17.3 |

Data adapted from Svejgaard et al. (68) and Tiwari and Terasaki (69). Calculation of relative risk is shown in Table 22.

**TABLE 22.** Calculation of relative risk: The 2 × 2 table

| | Number of individuals | |
|---|---|---|
| | Marker present | Marker absent |
| Patients | a | b |
| Controls | c | d |

$$\text{Relative risk} = \frac{a \times d}{b \times c}$$

## Mechanisms

Some of the disease associations with HLA do not involve the class I or class II MHC-encoded molecules. For example, congenital adrenal hyperplasia is associated with a defect in the enzyme 21-hydroxylase encoded in the HLA class III (complement) region. Similarly, C2 deficiency can be understood as a defect in a structural complement component. One of the most baffling associations of HLA with disease is the case of narcolepsy and HLA-DR2. This condition (the sudden, irresistible compulsion to sleep) is one of the tightest associations: in various studies 98 to 100% of narcoleptics carry the HLA-DR2 marker. Since there has never been any postulated involvement of the immune system or an infectious agent with narcolepsy, it seems likely in this instance that the association is not with an α,β class II MHC molecule but with an unknown linked gene.

The majority of diseases listed in Table 21 are strongly suspected to involve class I or class II molecules. They frequently show the involvement of autoantibodies and inflammatory cells such as in the cases of myasthenia gravis, lupus, rheumatoid arthritis, juvenile diabetes, and Goodpasture's syndrome. It would be comforting if we could recognize two broad categories of HLA-linked diseases. For example, in the class I-linked (HLA-A, B, C) diseases we might predict the presence of CTL responses, while the class II-linked (HLA-D) diseases might involve helper lymphocytes. In general, however, we cannot classify the diseases along these lines and in some instances the associations of one particular disease can be with class I *and* class II markers in different groups of individuals.

Two types of plausible etiologies follow from our understanding of MHC function:

1. An external agent (virus, bacterium) infects an individual. The specificity of the T cell response is dictated by the class I and class II molecules and the foreign peptides they present. The T cell response cross-reacts on a self-peptide plus MHC. According to this hypothesis, the preexisting self-complex was not in sufficient quantity, or at the correct location, to tolerize or to induce the involved T cells, but the self-complex can nevertheless serve as a target for effector cells induced by the cross-reactive invader. The T cells themselves may be directly responsible for the disease symptoms by attacking the tissues. In this case, for a class II-associated disease, the tissue would have to express class II molecules or supply the autoantigen to adjacent presenting cells. On the other hand, T helper cells cross-reactive with a determinant on a self-protein may stimulate a harmful autoantibody response to other determinants on the self-molecule, and antibody may be the ultimate cause of the disease. In a situation like this, the antibody itself would not be expected to cross-react with the foreign inducing agent.

2. In an updated version of the molecular mimicry hypothesis, an individual with a particular *HLA* haplotype would fail to mount a T cell response to a critical viral epitope because self-peptides plus self-MHC molecules have tolerized the T cells. In this case, the immune response may be delayed such that the infection would progress to ultimately cause the disease.

## CONCLUSION

The function of the class I and class II molecules encoded within the MHC is to bind peptide fragments derived by intracellular degradation of protein antigens. The peptide–MHC complexes are then presented at the cell surface to the T cell population, resulting in the phenomenon of MHC-restricted T cell recognition. T cells can be roughly categorized into one of two functional subsets, which are restricted to one or the other class of MHC molecules. Helper T cells recognize peptide antigen in association with self-class II molecules, whereas cytotoxic T cell recognition is class I restricted. Since both T cell subsets are specific for fragmented antigen presented by the MHC-encoded products, it is the role of intracellular processing and presentation mechanisms to discriminate between class I and class II presentation of antigen. It is currently believed that soluble, extracellular antigen is endocytosed and degraded almost exclusively within the lysosomal compartment of a cell, producing peptides for association with class II. Thus class II-expressing cells such as B cells and dendritic cells present soluble antigen to T helper cells. In contrast, proteins located within the cell cytoplasm, such as viral gene products or endogenous minor histocompatibility antigens, are thought to be degraded by an unknown cytosolic mechanism prior to presentation by class I MHC products. Thus all cell types that express class I can present endogenous antigens to cytotoxic T cells.

Extensive functional and biochemical analysis has characterized the class II MHC product, the Ia molecule, as a protein with a single peptide binding site which forms an extremely stable complex with a large number of immunogenic peptides. Each class II (and, by analogy, each class I) allelic molecule can bind a range of, but not all, immunogenic peptides. The ability to bind to Ia appears to be a prerequisite in determining whether a peptide is or is not immunogenic for an individual of a given MHC haplotype. A single binding site, in addition to stable peptide association, was also deduced from the crystallographic analysis of a class I MHC glycoprotein. Moreover, the crystal structure gives us the relative location of the various polymorphic residues that affect MHC function. Many of the residues appear to be in-

volved in determining the specificity of peptide binding and may not themselves contact the T cell receptor. Other polymorphic residues may interact directly with the T cell receptor.

The class I and class II molecules expressed on thymic epithelial cells are responsible for selecting the T cell repertoire during T cell differentiation. Only those immature thymocytes that express α,β T cell receptors with a certain minimum affinity for either the class I or the class II molecules expressed as self in the thymus are selected to mature. How this process of self or thymic selection comes about is not known. Thymocytes that pass this stage of positive selection are later tolerized to all self-MHC–self-peptide complexes. This step of negative selection may occur in a separate compartment of the thymus and may be performed most efficiently by bone-marrow-derived cells.

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